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LINKAGE BETWEEN PURPLE LEAF-SHEATH COLOUR AND JUICINESS OF STALK IN SORGHUM.

By G. N. Rangaswami Ayyangar, B.A., I.A.S.,
M. A. Sankara Ayyar, B.A., B.Sc.Ag.,
and
V. Panduranga Rao, M.A.
(*From the Agricultural Research Institute, Coimbatore.*)

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Sorghum leaf-sheaths are either purple in colour or brown (devoid of purple) (Fig. 1). Purple (PP) is a simple dominant to brown (pp) (Rangaswami Ayyangar, *et al.*, 1933).

Sorghum stalks may be pithy or juicy. When pithy, the midribs of the leaves have a white colour and when juicy, the midrib is of a dull leaden colour (Fig. 2). A pithy stalk, indicated by the white midrib of the leaf (DD), has been found to be a simple dominant to a juicy stalk with a dull midrib (dd) (Rangaswami Ayyangar, *et al.*, 1936).

The *Talai virichan cholam* of Coimbatore (*Sorghum Roxburghii* var. *hians* Stapf) is a coarse sorghum whose stalks are always pithy (juice extraction about 13%) and whose leaves have therefore white midribs. In this *Talai virichan cholam*, brown sheathed plants and varieties are met with in very large numbers.

The *Tella jonna* of Bellary belongs to the group *S. cernuum* Host and is typical of it. It is juicy with an extraction of about 40%. Its leaf has a markedly dull midrib. Its straw is reputed as fodder. Evolved in the black soils of the Deccan uplands this *Tella jonna* does not fare well under Coimbatore conditions. It was therefore designed to make crosses between *Talai virichan cholam* (*S. Roxburghii* var. *hians* Stapf) and the *Tella jonna* of Bellary (*S. cernuum* Host) with a view to tone up the fodder value of the former.

The following parents were chosen and were crossed—Cross No. A. S. CXXV.

No. of the parent	Name of variety	Colour of leaf-sheath	Colour of midrib of leaf
A.S. 171	*Tella jonna*	Purple	Dull
,, 1902	*Talai virichan cholam*	Brown	White

The first generation plants had purple leaf-sheaths and white midribs. In the second generation if there had been independent assortment of the factors for leaf-sheath colour and midrib colour, four groups of plants differing in leaf-sheath colour and midrib colour, *viz.*, purple sheath and white midrib, purple sheath and dull midrib, brown sheath and white midrib, and brown sheath and dull midrib, would have been obtained in the ratio of 9 : 3 : 3 : 1.

The F_2 generation however gave the following numbers in the four groups :—

Cross No. A.S. CXXV—F_2 Generation.

Family No.	Leaf-sheath →	Purple		Brown	
	Leaf midrib →	White	Dull	White	Dull
A.S. 3434	..	98	39	38	3
,, 3435	..	84	32	27	3
,, 3914	..	151	51	52	4
,, 3915	..	168	62	72	7
	Total ..	501	184	189	17
	Calculated (9 : 3 : 3 : 1)..	501·3	167·1	167·1	55·7

$\chi^2 = 31{\cdot}69$; $P > {\cdot}01$.

The high value of χ^2 shows that the distribution of the four groups does not conform to the normal di-hybrid ratio. Such a distribution of the four groups as that obtained above can be explained only on the assumption

FIG. 1.

Purple Brown

Leaf-sheath

FIG. 2.

White Dull

(Pithy stalks) (Juicy stalks)

Leaf midrib

of a linkage between the factor *P*, for purple leaf-sheath colour and the factor *D*, for juiciness of stalk (midrib colour).

On this assumption the recombination percentage was worked out and gave the value of 30 ± 1·8, in the repulsion phase.

On the assumption of linkage with a recombination percentage of 30, the expected distribution of the four groups of plants is as follows :—

Leaf-sheath →	Purple		Brown	
Leaf midrib →	White	Dull	White	Dull
Numbers obtained ..	501	184	189	17
Calculated (30% cross over).	465	203	203	20

$\chi^2 = 5 \cdot 98$; P > ·05.

The value $\chi^2 = 5 \cdot 98$ with P greater than ·05 shows that the assumption of linkage with a 30% cross-over satisfactorily explains this deviation from the simple di-hybrid ratio.

Plants with brown leaf-sheaths and dull leaf midribs gave a juice extraction of 40%.

Summary.

In sorghum there is a linkage between the factor *P* for purple leaf-sheath colour and *D* for juiciness of stalk, with a recombination percentage of 30±1·8.

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THE OCCURRENCE AND INHERITANCE OF WAXY BLOOM ON SORGHUM.*

By G. N. Rangaswami Ayyangar, B.A., I.A.S.,
V. Panduranga Rao, M.A., A. Kunhikoran Nambiar, B.Sc.Ag.,
and
B. W. X. Ponnaiah, B.Sc.Ag.
(*From the Agricultural Research Institute, Coimbatore.*)

Received December 21, 1936.

In many plants the effectiveness of the control exercised on transpiration by the epidermis is often enhanced by such adaptations as the secretions of a coating of wax, resin, etc. The characteristic "bloom" on grapes, apples, and plums as well as on the stems and leaves of many plants is due to the presence of such waxy coatings.

This deposit of wax may be in the form of closely crowded granules, or rods or needle-like structures. These rods or granules are deposited one upon the other in so crowded or intertwined a fashion as to form a fluffy covering over the epidermis. It has been experimentally proved that this waxy covering checks transpiration to a great extent (20 to 30 per cent.), Haberlandt (1928). It keeps off water which would clog the stomata and prevent the passage of gases, Ganong (1913). It also helps to keep off ants and other crawling insects from doing damage to the stem, flowers and fruits. As to the mode of formation of waxy covering Haberlandt has recorded that DeBary had shown that the wax did not, as might be supposed, arise by chemical modification of the cuticle or of other layers of the outer wall, but that it was on the contrary a product of secretion.

The physical structure, the chemical nature and the physiological functions of these waxy incrustations on plants and trees have been recorded [Haberlandt (1928) and Allott (1934)]. In sugarcane, Barber (1914) records the occurrence of the bloom and its marked and constant presence in the "bloom band" of the cane. His description of the distribution of this bloom in sugarcane is roughly parallel to that of sorghum.

The genetics of this character has been worked in a few cultivated plants, Matsuura (1933). Tschermak and Vilmorin (Matsuura, 1933) working on *Pisum* have separately reported that the wax covered (glaucous)

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was a simple dominant over the waxless (emerald) condition. Vilmorin in a cross between two emerald varieties got a glaucous F_1 and in the F_2 generation obtained a 9 : 7 ratio of glaucous to emerald. The crossing experiments of these workers and of Wellensiek (1925) indicate that two genes are involved in the inheritance of bloom. White (1918), Harland (1920 and 1922), and Peat (1928) have separately worked on this character in *Ricinus* and have reported that three pairs of genes are responsible for the inheritance of bloom in its various grades. The bloomed condition is dominant over the bloomless. In *Rubus*, Wellington (Matsuura, 1933) has reported that the bloomed condition is partially dominant, the glaucous canes giving glaucous, intermediate and non-glaucous ones in the ratio of 1 : 2 : 1. In *Brassica*, Kajanus (Matsuura, 1933) has found that the waxy covering of the leaf is partially dominant over the waxless condition, the F_2 individuals being continuous between the extreme or parental types, giving a 1 : 2 : 1 ratio. In *Pirus*, Wellington (Matsuura, 1933) found that the abundance of bloom on the stem is recessive to little or no bloom. In *Prunus*, Wellington (Matsuura, 1933) has shown from certain crosses that thick bloom on the fruit is dominant over thin bloom. In *Bryonia*, the presence of bloom on the berry has been reported to be completely recessive to its absence by Jones and Rayner (1916). In *Salix*, Heribert-Nilsson (Matsuura, 1933) has shown that "the blue waxy condition of *S. daphnoides* was dominant to the non-waxy of *S. purpurea*, while in a cross with the non-waxy *S. viminalis* the character of *S. daphnoides* proved to be recessive. The latter cross gave in the F_2 several gradations of waxy covering." In *Canna*, Honing (Matsuura, 1933) has reported the existence of polymeric genes for waxy layers on the leaves, the bloomed condition in combination with other characters being dominant over the bloomless. In *Triticum*, according to Tschermak (Matsuura, 1933) Miczinsky obtained results indicating that the wax formation on the leaf surface is dominant to the smooth green leaf. But Biffen (1916) records the appearance of plants with green leaves among the offspring of the cross between two waxy types. In *Avena*, Nilsson-Ehle (Matsuura, 1933) has shown that the presence of bloom on the lemma is partially dominant over its absence. It was further found that the formation and degree of wax efflorescence was also due to another (possibly one) strengthening gene the absence of which resulted in a relatively thin deposit. In *Secale*, Tschermak and Heribert-Nilsson (Matsuura, 1933) working separately reported that the presence of wax on the stem, leaves and glumes was dominant to its absence. It will thus be seen that in the majority of cases reported the bloomed condition is dominant to its partial or complete absence.

Sorghum, the giant millet, is mostly raised under rain-fed conditions. Rains are apt to fail or be sparse. Though both drought resistant and drought evasive, the sorghum crop with its heavy foliage runs the risk of drying, if the heavy transpiration due to the high tropical temperature is not checked. To control this high rate of transpiration the epidermis secretes a lot of wax which forms a covering for the plant and reduces transpiration by partly closing up the stomata. It is common to observe when the temperature is high during the middle of the day, that the leaves of the sorghum plant fold in along the midrib or roll up covering the top surface and exposing the under surface with its heavy protective coating of wax. The importance of this adaptation is obvious.

All sorghum types so far examined have a very light basic layer of wax on the leaves (on both sides) which is not visible to the naked eye. It is on this wax that dust, soil particle or other foreign matter sticks. Scrapings from the surfaces of leaves when examined under the microscope consist of small particles of soil mixed up with granules of wax. This wax is in the form of lumps with irregular shapes and not in the form of fibres. In certain types such as broom corns (*Andropogon Sorghum* var. *technicum*) and many species of wild sorghums, this type of covering is fairly thick and on a dark green background imparts a bluish green look to the plant.

A more conspicuous waxy covering visible even to the naked eye is found over this basic layer in all sorghums examined so far and its distribution is heavy in some and sparse in others. This latter type is in the form of fibres. It covers the former and gives an ashy look to the plant. It is when both the kinds of secretions are markedly sparse that the plant presents a glossy look.

An examination of both cultivated and wild sorghums of Asiatic or African origin shows that this waxy secretion is found in all in some degree or other. Forms that appear to be bloomless reveal traces thereof on careful examination. Types completely free from this bloom have not been met with so far. The tropical sorghums have a much thicker coating of wax than the sub-tropical forms. The African types and the African introductions into America have a heavy bloom while the Chinese *kaoliangs* have comparatively less bloom. The Indian types, particularly those from Deccan and the Central Provinces, are more or less heavy bloomed. Under normal conditions the irrigated types produce less waxy secretion than the rain-fed ones.

This waxy secretion begins to appear very early in the life of the plant. The examination of seedlings at various stages of growth shows that the

secretion can be made out with a lens even on the third or fourth day after germination. In seedlings a week old, enough visible deposit is formed on the leaf-sheath and the lower surface of the leaf. As the seedling grows, the secretion of wax increases and the incrustation becomes thicker, and is most in evidence as the plant approaches the flowering phase, when the rate of secretion is highest. Thereafter there is a gradual decline.

As already recorded the leaf-sheath and the lower surface of the leaf are the regions which show the earliest traces of the deposit. When the internodes are formed, the bloom is seen on the internodes also, more prominently on the exposed regions, and particularly so at the very top of the internode. Later on with the emergence of the boot and of the head, the glumes, the central axis and the bases of panicle branches show traces of this waxy secretion. In no region is it so thick as on the boot, the final leaf-sheath. Whether on the leaf-sheath, leaf-blade, or on any other region, the deposit is more pronounced on the intravenous region. The upper surface of the leaf is free from this deposit. As the plant grows, there is a gradual fading of this waxy bloom from the lower leaf-sheaths, internodes and leaves and a more or less heavy deposit on the covered internodes which becomes visible when the leaf-sheath is pealed off from a well-grown plant.

The bloom is made up of a characteristic tangle of thin cellular fibre or lint-like structures of wax spread over the epidermis. The secretion which forms this bloom exudes through the pores of the cells of the epidermis. The cells continue to secrete and the wax gets deposited on the epidermis in the form of long thin fibre-like formations. Microscopic examination of a bit of the waxy covering shows that it consists of numerous bits of threadlike formations which are hooked or rolled up at their blunt, distal ends (Fig. 1). Under reflected light they are white to very light bluish white in colour. They have an average diameter of 3 to 4 μ and length of 100 to 150 μ. Many of these are longer than the epidermal cells. The deposit is comparatively heavier round about the stomata. With the increase in the deposit they get crowded and interwoven and give the appearance of a tangle of hooked fibres.

The earliest deposits consist mostly of shorter fibres (30 to 40 μ long), while the later ones contain short and long ones mixed up. The deposits from mature plants are made up mostly of long fibres only and this indicates that the secretion of wax slows down very much after flowering and seed setting. Unless removed mechanically the waxy incrustation, once formed, sticks on the plant and will remain there long even after the plant is dead, dry stalks with the remnants of this bloom being commonly seen. In old stalks exposed to weathering the waxy secretion is not seen in the form of

long fibres, but as broken bits or granules. Further exposure results in the practical disappearance of the bloom.

In the thickly deposited condition the waxy covering gives the plant a heavily powdered look (Fig. 2). It is this markedly powdered look of some varieties that has led to such characteristic varietal names as *Vibhuthi Jonna* (sacred-ashes sorghum).

Wax in the form of granules or fibre-like structures is easily wiped off by rain or other mechanical agencies. It is therefore a disadvantage to the plant, if it could not regenerate the waxy coating that has been removed. In sorghum, as in many other plants with bloom, this waxy covering, if removed, is quickly replaced by fresh secretion. Weather continuing fine, when the bloom was removed at the flowering period, the plants regenerated the bloom and restored their former appearance in about three days. This power of regenerating a waxy secretion, as long as such a protection is required, is a valuable asset to this premier cereal of the dry tract.

The waxy secretion gathered from the plant was supplied to the Agricultural Chemist who examined it. He reported that the sample was a white powdery substance and was absolutely tasteless and odourless, that on treating with alcohol, ether or chloroform, it showed it to be a wax, and that its melting point was 83 to 84° C. Staining tests also show that it is a wax.

All sorghum varieties have this bloom in some degree or other. The degree of manifestation is of two different types—heavy and sparse. Both the heavy and sparse bloomed varieties exist in many groups of sorghum, though in certain groups the heavy bloomed condition is marked. Kafir (*Sorghum caffrorum*, Beauv.), Milo (*Sorghum caudatum*, Stapf var. *milo*) and Feterita (*Sorghum caudatum*, Stapf var. *feterita*), grown at Coimbatore are all heavily bloomed. In the above groups it is so thick that the bloom will come off in flakes. The group *Sorghum cernuum*, Host, is also markedly bloomy. Many heavily bloomed varieties occur in *Sorghum Roxburghii* var. *hians*, *Sorghum nervosum*, Bess., and *Sorghum margaritiferum*, Stapf. In the major grain sorghum group *Sorghum Durra*, Stapf, owing to its wide range in origin and cultivation, there is an equal distribution of heavy bloomed and sparse bloomed varieties. *Sorghum papyrascens*, Stapf, is generally heavy bloomed. *Andropogon Sorghum*, var. *technicum* (Koern.) Bailey, and *Andropogon Sorghum* var. *saccharatus*, Koern., as well as *Sorghum guineense*, Stapf, are generally sparse bloomed. In the wild sorghums the following groups, *viz.*,—*Sorghum versicolor*, J. N. Anderss, *Sorghum dimidiatum*, Stapf, *Sorghum purpureosericeum*, Aschers and Schweinf, *Sorghum*

virgatum, Stapf, *Sorghum verticilliflorum*, Stapf, *Sorghum halepense* (Linn.) Pers., and *Sorghum sudanense*, Stapf, show a distribution that is more sparse than in the grain sorghums.

The degree of manifestation, as recorded, is of two different types—heavy and sparse. In the heavy condition the plant has a distinct ashy look. There is a concentration of bloom in the leaf-sheath, especially on the boot, and on the internode. It is extra-heavy at the tops of both the

TABLE I.

Segregations for Waxy Bloom (Heavy and Sparse).

Selection No.	Parents		F_1 Heavy bloomed	F_2 Segregation	
	Heavy bloomed	Sparse bloomed		Heavy bloomed	Sparse bloomed
CLVII	M.S. 1404	A.S. 809	Heavy bloomed	76	28
CLIX	,,	,,	,,	25	8
A.S. 3727	A.S. 2876	A.S. 818	,,	56	18
A.S. 4386	A.S. 1751	A.S. 2157	,,	222	71
A.S. 4387	,,	,,	,,	308	114
A.S. 4389	A.S. 1745	,,	,,	446	151
A.S. 4390	,,	,,	,,	52	19
A.S. 4391	A.S. 3464	A.S. 367	,,	142	40
A.S. 4392	,,	,,	,,	136	36
A.S. 4393	,,	,,	,,	137	45
A.S. 4394	,,	,,	,,	186	63
A.S. 4395	,,	,,	,,	146	56
A.S. 4396	,,	,,	,,	150	61
			Total ..	2082	710
Expected (on 3 : 1 basis)				2094	698

$\chi^2 = \cdot 27$; $\cdot 7 > P > \cdot 5$.

leaf-sheath and internode. In the heavy type the whole under-surface of the leaf-blade is bloomy. In the sparse condition this markedly ashy look is not presented. A close examination will show the waxy deposit at the critical places, namely, the top of the leaf-sheath, the top of the internode, and the base of the under-surface of the lamina. The separation into heavy and sparse is well marked, there being no blended intermediates on crossing. These heavy and sparse bloomed conditions have proved a pair of simple Mendelian characters.

The inheritance of this waxy secretion was studied in four artificial crosses. Table I gives segregations for the heavy and the sparse bloomed conditions.

Other experiences in this segregation are recorded in Table II.

TABLE II.

Segregations for Waxy Bloom.

Selection No.	F_2 Segregation	
	Heavy bloomed	Sparse bloomed
A.S. 3366 ..	119	40
A.S. 3791 ..	97	24
A.S. 3793 ..	152	50
A.S. 3949 ..	49	17
A.S. 3957 ..	43	13
A.S. 3958 ..	47	15
A.S. 3964 ..	18	6
A.S. 4065 ..	95	29
Total ..	620	194
Expected (on 3:1 basis) ..	610·5	203·5

$\chi^2 = \cdot 59 : \quad \cdot 5 > P > \cdot 3.$

An F_3 was raised from A.S. 3949 and behaved as in Table III.

TABLE III.

F_3 Generation from A.S. 3949.

Selection No.	Character of Selection	F_3 Segregation	
		Heavy bloomed	Sparse bloomed
A.S. 4812 ..	Heavy bloom	Pure	..
A.S. 4821 ..	,,	Pure	
A.S. 4823 ..	,,	Pure	
A.S. 4827 ..	,,	Pure	
A.S. 4814 ..	Sparse bloom	..	Pure
A.S. 4815 ..	,,	..	,,
A.S. 4818 ..	,,	..	,,
A.S. 4819 ..	,,	..	,,
A.S. 4820 ..	,,	..	,,
A.S 4824 ..	,,	..	,,
A.S. 4831 ..	,,	..	,,
A.S. 4832 ..	,,	..	,,
A.S. 4835 ..	,,	..	,,
A.S. 4813 ..	Heavy bloom	72	32
A.S. 4816 ..	,,	90	23
A.S. 4817 ..	,,	50	19
A.S. 4822 ..	,,	102	28
A.S. 4825 ..	,,	82	19
A.S. 4826 ..	,,	66	22
A.S. 4828 ..	,,	63	21
A.S. 4829 ..	,,	63	22
A.S. 4830 ..	,,	55	17
A.S. 4833 ..	,,	66	24
A.S. 4834 ..	,,	91	25
A.S. 4836 ..	,,	46	11
Total ..		846	273
Expected (on 3 : 1 basis) ..		839·25	279·75

From the above tables it will be seen that the segregation is monogenic and that the heavy bloomed condition is dominant to the sparse bloomed condition. A factor H produces the heavy bloomed condition. In its absence (h) the plants secrete only a sparse bloom. H is a simple dominant to h.

The relationship between the characters heavy bloomed and sparse bloomed and other plant characters was examined. The sheath is the place

at which the bloom is easiest to detect. Sheaths (and glumes) can be either reddish purple (PQ), blackish purple (Pq), or brown in colour (pQ or pq). (Rangaswami Ayyangar, *et al.*, 1933.) The relationship between the factors H and P and Q was gone into and the Tables IV and V show their independent inheritance.

TABLE IV.

Heavy Bloom (H) and Leaf-Sheath and Glume Colour (Q).

Selection No.	Heavy bloomed (H)		Sparse bloomed (h)	
	Sheath RP (PQ)	Sheath BP (Pq)	Sheath RP (PQ)	Sheath BP (Pq)
A.S. 3958 ..	36	11	12	3
Expected (9 : 3 : 3 : 1)	34·7	11·7	11·7	3·9

$\chi^2 = \cdot 31$; $\cdot 98 > P > \cdot 9$.

RP = Reddish Purple; BP = Blackish Purple; Br = Brown.

TABLE V.

Heavy Bloom (H) and Leaf-Sheath and Glume Colour (P).

Selection No.	Heavy bloomed (H)		Sparse bloomed (h)	
	Sheath BP (Pq)	Sheath Br (pq)	Sheath BP (Pq)	Sheath Br (pq)
A.S. 3949 ..	39	10	14	3
Expected (9 : 3 : 3 : 1)	37·3	12·3	12·3	4·1

$\chi^2 = 1 \cdot 04$; $\cdot 8 > P > \cdot 7$.

It was noted that in the heavy bloomed types the under-surface of the leaf was heavily bloomed. From Table VI it will be seen that the dispositions of the margin of the leaf-blade whether undulated (Mu) or flat (mu) (Rangaswami Ayyangar, *et al.*, 1935) have no selective influence on the bloomed conditions heavy (H) or sparse (h).

TABLE VI.

Heavy Bloom (H) and Leaf Margin Disposition (Mu).

Selection No.	Heavy bloomed (H)		Sparse bloomed (h)	
	Leaf Margin Undulated (Mu)	Leaf Margin Flat (mu)	Leaf Margin Undulated (Mu)	Leaf Margin Flat (mu)
A.S. 3366 ..	87	32	31	9
Expected (9 : 3 : 3 : 1)	89·7	29·7	29·7	9·9

$\chi^2 = .39$; $\cdot 95 > P > \cdot 9$.

It has been noted in another article (G. N. Rangaswami Ayyangar, *et al.*, 1934) that a chalky condition of the grain surface adapted it to lighter loams. To find out any possible relationship between this adaptation and bloom, crosses were designed and the following segregations (Table VII) show that the character pair H — h is independent in inheritance of the pair Z — z.

TABLE VII.

Heavy Bloom (H) and Grain Surface Structure (Z).

Selection No.	Heave bloomed (H)		Sparse bloomed (h)	
	Pearly (Z)	Chalky (z)	Pearly (Z)	Chalky (z)
A.S. 3965 ..	55	16	13	6
Expected (9 : 3 : 3 : 1)	50·8	16·8	16·8	5·6

$\chi^2 = 1 \cdot 28$; $\cdot 8 > P > \cdot 7$.

The relationship between the H—h characters and the B factors determining the brown grain was gone into and Table VIII will show that they are independenc in inheritance.

TABLE VIII.

Heavy Bloom (H) and Grain Colour Factors B_1 and B_2.

Selection No.	Heavy bloomed (H)		Sparse bloomed (h)	
	Brown grain B_1B_2	White grain B_1b_2, b_1B_2, or b_1b_2	Brown grain B_1B_2	White grain B_1b_2, b_1B_2, or b_1b_2
A.S. 4064 ..	39	31	20	13
A.S. 4065 ..	55	40	18	11
Total ..	94	71	38	24
Expected on (27 : 21 : 9 : 7 basis) ..	97·5	73·5	31·5	24·5

$\chi^2 = 1{\cdot}57$; $\cdot 7 > P > \cdot 5$.

It will thus be seen that the waxy bloom character pair H—h is independent in inheritance to the leaf-sheath colour characters RP, BP and Br. (P and Q) leaf-margin disposition characters—undulated and flat (Mu—mu), the grain surface structure characters pearly and chalky (Z—z), and the B factors responsible for the manifestation of brown colour on the grain and anthers (B_1 and B_2).

Summary.

All sorghum varieties develop a waxy bloom. When heavy this bloom is present on the leaf-sheath, leaf-blade, internode, panicle branch and glume. It is best seen at flowering time. When sparse, the bloom is easily seen at the top of the leaf-sheath, top of the internode, and the base of the under-surface of the leaf. Some groups of sorghum show the bloom in heaviness and others in sparseness. The heavy bloomed condition (H) is a simple dominant to the sparse bloomed condition (h). The factor (H) is independent in inheritance to the leaf-sheath colour factors P and Q, leaf margin disposition factor Mu, grain surface structure factor Z, and the brown grain factors B_1 and B_2.

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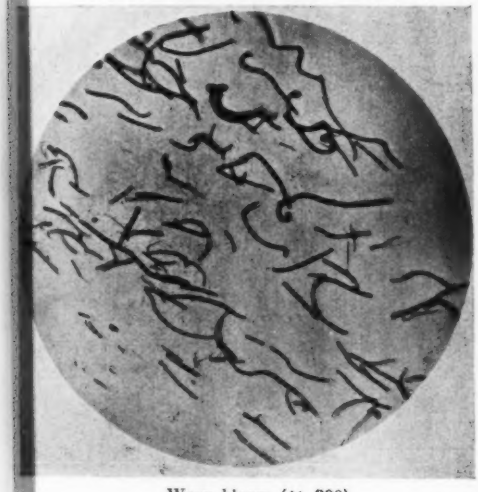
FIG. 1.



Waxy bloom (× 300)

FIG. 2.

Heavy bloomed. Sparse bloomed.

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THE CHLOROPHYCEÆ OF THE BOMBAY PRESIDENCY, INDIA—I.

By S. C. Dixit, M.A., M.Sc.,
Wilson College, Bombay.

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[Communicated by Prof. Y. Bhâradwâja, M.Sc., Ph.D. (Lond.), F.L.S.]

The Presidency of Bombay is well known for its varied climatic and topographical features and the whole province is far from what may be called a 'homogeneous province' more especially from the botanical point of view. It possesses all types of vegetation ranging between the Arabio-African flora in the North and the Indo-Malayan one in the South. The study of the algæ collected from various localities would, therefore, be of great interest, and it is with this object that the writer has planned to work out the algal flora of the Bombay Presidency. In a previous communication[1] some forms of Myxophyceæ collected from Bombay were described, but in this paper the writer gives a systematic account of some of the fresh-water Chlorophyceæ gathered from Bombay and its environs during the last five years. It may be mentioned that, except for some marine forms recently described by Boergesen, there is no record of green algæ from this part of the country.

Observations made at different localities have shown that a number of marine Chlorophyceæ have migrated into fresh-water and a few fresh-water forms are inhabiting brackish-water areas. Among the former may be mentioned, (1) *Enteromorpha prolifera* J. Ag., (2) *Chætomorpha indica* Kütz., (3) *Rhizoclonium Kochianum* Kütz., and (4) *Vaucheria piloboloides* Thur., while (1) *Hydrodictyon reticulatum* (L.) Lagerheim, (2) *Rhizoclonium hieroglyphicum* (C. A. Ag.) Kütz., and (3) *Stigeoclonium lubricum* Kütz. are of the latter type.

In all thirty-six forms have been described in this paper out of which four have been found growing on soil, three on stones or rocks, one on a gastropod shell, four in running water and the rest from the steady waters of pools, tanks and ponds. There are two new species and one new variety. Some species of *Euglena*, *Mougeotia* and *Debarya*, although collected in large quantities, could not be identified owing to the materials being sterile.

[1] Dixit, "The Myxophyceæ of the Bombay Presidency, India—I," *Proc. Ind. Acad. Sci.*, Vol. III, No. 1, (B), 1936.

SYSTEMATIC ENUMERATION OF THE SPECIES OBSERVED.*

Class Chlorophyceæ.
Order Volvocales.
Sub-order Chlamydomonadineæ.
Family Sphærellaceæ.
Genus *Volvox* (L.) Ehrenberg.

1. *Volvox dissipatrix* (Shaw) Iyeng. Iyengar, "Contributions to Our Knowledge of the Colonial Volvocales of South India," *Journ. Linn. Soc. Bot.*, XLIX, 1933, p. 356.

Fam. Sex., 1,000–1,380 μ; protoplastis, 5–6 μ lat.; oosp., 37–40 μ lat.

Habitat.—Dudhia Talao, Pavagadh, Gujerat (about 2,000 ft. above sea-level). (November.)

Order Chlorococcales.
Family Chlorococcaceæ.
Genus *Characium* A. Braun.

2. *Characium ambiguum* Hermann. Lemmermann, Brunnthaler und Pascher in Pascher's *Süsswasser-Flora Deutschlands, Österreichs und Der Schweiz.*, Heft 5, 1915, Fig. 17.

Forma.

Lat. Cell., 4–7·4 μ; long. cell., 22–26 μ.

Habitat.—Epiphytic on a sterile species of *Pithophora*, Borivali. (August.)

This form is slightly shorter than the type.

Family Hydrodictyaceæ.
Genus *Pediastrum* Meyen.

3. *Pediastrum duplex* Meyen. Var. *regulosum* Raciborski. Lemmermann, Brunnthaler und Pascher, *op. cit.*, 1915, Fig. 57 *k*.

Colon., 16–32 cells; crass. colon., 40·7–85·5 μ; lat. cell., 11–14·8 μ.

Habitat.—In the Laboratory fresh-water aquarium, Wilson College, Bombay. (February.)

4. *Pediastrum tetras* (Ehrenb.) Ralfs. Ralfs, *The British Desmidieæ*, 1848, Pl. XXI, I; Fritsch and Rich, "Fresh-water Algæ of Africa," *Trans. Roy. Soc.*, South Africa, 1924, XI, p. 311, Fig. 1, *D-F*.

Colon., 8 cells; crass. colon., 29·6–33·3 μ; lat. cell., 5·4–7·4 μ.

Habitat.—In a pond, Jogeshwari. (September.)

* The classification given by F. E. Fritsch, in *The Structure and Reproduction of the Algæ*, 1, Cambridge, 1935, has been followed in this paper.

The forms observed certainly belong to *Pediastrum tetras* (Ehrenb.) Ralfs, but there is a variability in the shape of the incision between the marginal processes, as has been observed by Fritsch and Rich. All the three forms drawn by them have been met with.

Genus *Hydrodictyon* Roth.

5. *Hydrodictyon reticulatum* (L.) Lagerheim. Lemmermann, Brunnthaler und Pascher, *op. cit.*, 1915, Fig. 68.

Lat. cell., 83–148 μ ; long. cell., 5 mm.

Habitat.—In pools, rivulets and rarely in brackish-water, mixed with *Enteromorpha prolifera* J. Ag., 1. Borivali, 2. Poona. (September.)

Family Cœlastraceæ.

Genus *Scenedesmus* Meyen.

6. *Scenedesmus ecornis* (Ralfs) Chod. Ralfs, *The British Desmidieæ*, 1848, Tab. XXXI, Fig. 12 *h* ; Chodat, *Scenedesmus, De la Revue d'hydrologie*, III, Anne, No. 3/4. 1926, p. 170.

Colony of 2–4 cells ; lat. colon., 11–14·8 μ ; long. cell., 5·4–11 μ.

Habitat.—In a rainpool, Borivali. (September.)

7. *Cœlastrum microporum* Nægeli (= *Cœlastrum indicum* Turner). Pascher, *op. cit.*, 1915, Fig. 307 ; Smith, " The Plankton Algæ of the Okoboj Region, " *Trans. Amer. Mic. Soc.*, 1926, XLV, 3, Pl. 18, Figs. 10–11.

Lat. cell., 7·4–18·5 μ.

Habitat.—In a rainpool, Borivali. (July.)

Order Ulotrichales.

Sub-order Ulotrichineæ.

Family Ulotrichaceæ.

Genus *Ulothrix* Kütz.

8. *Ulothrix variabilis* Kütz. Heering in Pascher's *Süsswasser-Flora Deutschlands, Österreichs und Der Schweiz.*, Heft 6, 1914, Fig. 27.

Lat. cell., 5–7 μ ; long. cell., 8–10 μ.

Habitat.—Epiphytic on a species of *Ceratophyllum* in ditches, Khar. (July.)

9. *Ulothrix zonata* Kütz. Heering, *op. cit.*, 1914, p. 35, Figs. 35 *a* and 36.

Lat. cell., 18·5–37 μ ; long. cell., 7·4–18·5 μ.

Habitat.—Epiphytic on aquatic grass in a pond, Bandra. (September.)

Family Ulvaceæ.

Genus *Enteromorpha* Link.

10. *Enteromorpha prolifera* J. Ag. Heering, *op. cit.*, 1914, p. 27, Fig. 24.

Lat. cell., 8–14 μ ; long. cell., 18·5–22·2 μ.

Habitat.—From seacoast and also river, Borivali. (August.)

Order Cladophorales.

Family Cladophoraceæ.

Genus *Chætomorpha* Kütz.

11. *Chætomorpha indica* Kütz. Boergesen, " A list of Marine Algæ from Bombay," Danske Videnskabernes Selokab, *Biologiske Meddelser Copenhagen*, 1935, XII, 2, p. 12, Fig. 2.

Lat. cell., 40–60 μ ; long. cell., 55–85 μ.

Habitat.—In Poisar River, Borivali ; in salt-pans, Govari (January.)†

Genus *Rhizoclonium* Kütz.

12. *Rhizoclonium Kochianum* Kütz. Boergesen, *Mar. Alg. D.W.I.*, Vol. I, p. 19, Fig. 7.

Lat. cell., 12·3–18·5 μ ; 1–2 times as long as broad.

Habitat.—In brackish-water, Thana (September) ; on mud-flat, Santa Cruz. (August.)

13. *Rhizoclonium hieroglyphicum* (C.A.Ag.) Kütz. Heering in Pascher's *Süsswasser-Flora Deutschlands, Österreichs und Der Schweiz*, Heft 7, 1921, Figs. 11, 13, 20 and 21.

Lat. cell., 12–31 μ ; long. cell., 37–129 μ.

Habitat.—On garden soil, Wilson College, Bombay. (November.)§

Genus *Cladophora* Kütz.

14. *Cladophora crispata* (Roth.) Kütz. ampl. Brand./Heering, *op. cit.*, 1921, p. 40, Fig. 40.

Crass. main fil., upto 45 μ ; crass ultimate branches, 25 μ.

Habitat.—On shells of living *Ampularia* sp. in tanks and ponds, Bandra, Borivali. (August-September.)

The alga completely agrees with the description given by Heering except that in certain specimens the production of two branches from the same cell was quite common.

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† This plant was previously collected from the sea-shore (*cf.* Boergesen, *op. cit.*, 1935, p. 12).

§ Recorded by Carter from Dadar, Bombay (*cf.* " Fresh-water Algæ from India," *Records Bot. Surv. Ind.*, 1929, 9, No. 4, Calcutta).

Genus *Pithophora* Wittrock.

15. *Pithophora polymorpha* Wittrock. Wittrock, *Pithophoraceæ*, Upsala, 1877, p. 62, Pl. I, Figs. 13–17 ; Pl. IV, Fig. 19.

Lat. fil., 80–100 μ ; crass. akin., upto 100 μ ; long. akin., upto 166 μ.

Habitat.—In ponds and ditches, Santa Cruz, Dadar.‡ (September–October.)

Order Chætophorales.

Family Chætophoreæ.

Genus *Stigeoclonium* Kütz.

16. *Stigeoclonium tenue* Kütz. Fritsch, *The Structure and Reproduction of the Algæ*, Vol. I, 1935, Fig. 74 *a* ; Butcher, " Little known Algæ from the Beds of Rivers," *New Phytologist*, Vol. XXXI, 5, 1932, p. 300, Fig. 6 *a*.

Lat. cell., 7·4 μ ; long. cell., 7·4–14·8 μ.

Habitat.—On stones in a rain-pool, Race course, Mahalaxmi ; in a pond, Victoria Gardens. (August.)

17. *Stigeoclonium lubricum* Kütz. Heering, *loc. cit.*, 1914, Heft 6, Fig. 114.

Forma salina form. nov.

Crass. main fil., 14·8–18·5 μ (rarely upto 25.9 μ) ; crass. branch fil., 7·4–11 μ.

Habitat.—On rocks in sea-water pools, Mahalaxmi Battery point.

The form differs from the type in possessing thicker branches and occasionally thicker main filaments. It further differs in growing in sea-water.

Genus *Chætophora* Schrank.

18. *Chætophora elegans* (Roth.) Agardh. Heering, *op. cit.*, Heft 6, 1914, Figs. 137 and 138.

Lat. cell., 5·4–7·4 μ ; 2–7 times as long as broad.

Habitat.—Epiphytic on aquatic plants, in a forest pool forming mucilaginous round masses, Jogeshwari. (September.)

Family Pleurococcaceæ.

Genus *Pleurococcus* Meneghini.

19. *Pleurococcus Nægeli* Chodat. Fritsch, *loc. cit.*, 1935, Fig. 90, *d* and *e*.

Lat. cell., 7·4–16·6 μ.

Habitat.—On walls, Elphinstone College, Bombay. (August–September.)

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‡ Carter (*op. cit.*, 1926, p. 278) records a form from Dadar, Bombay, under the name of *Pithophora radians* W. & G. S. West, but she examined only the sterile material. It is likely that her form may be the one described here, as the locality is just the same.

Order Oedogoniales.
Family Oedogoniaceæ.
Genus *Oedogonium* Link.

20. *Oedogonium plagiostomum* Wittrock. var. *gracilis* Wittrock. Hirn, "Morphologie und Iconographie der Oedogoniaceen," *Acta. Soc. Sci.*, Fenn. 27, 27, No. 1, 1900, p. 101, Pl. VI, Fig. 4 ; Tiffany, *The Oedogoniaceæ*, Ohio, 1930, Pl. XIV, Figs. 141 and 142.

Lat. cell., 22–26 μ ; long. cell., 55–64 μ ; oogonium 37–44 $\mu \times$ 55–60 μ ; crass. oosp. 37 μ ; long. oosp. 40–44 μ.

Habitat.—In a pool, Andheri. (July.)

The form differs from the type in having slightly shorter cells.

21. *Oedogonium Wolleanum* Wittrock. Hirn, *op. cit.*, 1900, p. 220, Pl. XXXVII, Fig. 226 ; Tiffany, *op. cit.*, 1930, Pl. L, Figs. 479 and 480.

Lat. cell. veg., 22–37 μ ; crass. cell. suffult, 55 μ ; crass. oog., 70 μ ; long. oog., 77 μ.

Habitat.—Epiphytic on aquatic grasses in a pond, Thana. (December.)

Genus *Bulbochæte* Agardh.

22. *Bulbochæte tenuis* (Wittrock.) Hirn. Hirn, *op. cit.*, 1900, p. 368, Pl. LXIII, Fig. 388 ; Tiffany, *op. cit.*, 1930, Pl. VII, Fig. 59.

Lat. cell., 11–14·8 μ ; long. cell., 22–33 ·3 μ ; crass. oog., 22–25 μ ; long. oog., 37–40 μ.

Habitat.—Epiphytic on *Zygnema* sp. in a water course, Mahabaleshwar. (January.)

Order Conjugales.
Sub-order Euconjugateæ.
Family Zygnemaceæ.
Genus *Zygnema* Agardh.

23. *Zygnema cyanosporum* Cleve. Czurda in Pascher's *Süsswasser-Flora Mittleeuropas*, Heft. 9, Jena, 1932, p. 106, Fig. 104.

Lat. cell., 18–22 μ ; long. cell., 40–60 μ ; crass. zygosp., 33–40 μ.

Habitat.—In a pond, Santa Cruz. (December.)

Genus *Spirogyra* Link.

24. *Spirogyra gracilis* (Kütz.) Czurda. Czurda, *op. cit.*, 1932, p. 172, Fig. 176.

Var. *magnum* Var. Nov. (Figs. 1 and 2).

Lat. cell., 33–37 μ ; long. cell., 74–116 (–259) μ ; crass. zygosp., 33–44 μ ; long. zygosp., 41–70 μ.

Habitat.—In ditches near Mâhakâl Caves, Andheri. (August.)

The variety differs from the type in the bigger size of the cells and broader zygospores possessing a bluish mesospore without any rib-line.

25. *Spirogyra setiformis* (Roth.) Kütz. Czurda, *op. cit.*, 1932, Fig. 202.

Forma.

Chromatophores, 8–10 ; lat. cell., 92–111 μ ; long. cell., 92–129 (–155) μ ; crass. zygosp., 66–74 μ ; long. zygosp., 92–118 μ.

Habitat.—In a pool mixed with *Hydrodictyon reticulatum* near Mt. Pazir, Borivali. (September.)

The form differs from the type only in the thinner mesospore.

26. *Spirogyra ellipsospora* Transeau. Curda, *op. cit.*, 1932, p. 203, Fig. 217.

Chromatophores, 4–6 ; lat. cell., 148–166 μ ; long. cell., 203–240 μ ; lat. zygosp., 100–111 μ ; long. zygosp., 155–174 μ.

Habitat.—In a stream near Canary Caves (Salsette). (V. N. Hate, January.)

27. *Spirogyra bimorphis* Sp. Nov. (Figs. 3–5).

Vegetative cells cylindrical, 2–3 times as long as broad ; end-walls plane. Chloroplasts, six ; conjugation scalariform ; fructifying cells not swollen. Zygospores ellipsoidal to spherical. Exospore thin, smooth and colourless ; mesospore thick, brown and sculptured in a reticulate manner ; endospore indistinct.

Lat. cell., 83–104 μ ; long. cell., 182–215 μ ; crass. zygosp., 81–103 μ ; long. zygosp., 111 μ.

Habitat.—In a pond, Alibaug near Bombay. (August.)

The alga belongs to the section Conjugata on account of the plane cross-walls (Czurda, *op. cit.*, 1932, p. 135). It can be compared with *Spirogyra paraguayensis* Borge and *S. Malmeana* Hirn, on account of the scalariform conjugation, unswollen fructifying cells and the ellipsoidal spores with a thick brown mesospore, but differs from both in having broader cells with six chromatophores and much bigger zygospores with the mesospore reticulately sculptured.

Genus *Sirogonium* Kütz.

28. *Sirogonium sticticum* Kütz. (= *Spirogyra stictica* Wille) Czurda, *op. cit.*, 1932, p. 144, Fig. 142.

Lat. cell., 38–55 μ ; lat. zygosp., 55–74 μ; long. zygosp., 75–88 μ.

Habitat.—In a rivulet, Goregaon. (September.)

29. *Sirogonium inflata* Sp. Nov. (Fig. 3).

Vegetative cells cylindrical ; end-walls plane. Chloroplasts 10, straight or taking half a turn. Fructifying cells very much inflated. Zygospores ellipsoidal with rounded ends or rarely ovoid. Exospore thin, smooth and colourless ; mesospore thin, brown and verrucose.

Lat. cell., 81–99 μ ; long. cell., 166–298 (–332) μ ; crass. zygosp., 81–85 μ ; long. zygosp., 111–139 μ ; crass. gametangia, 166–332 μ.

Habitat.—In a rain-pool, Petlad, North Gujerat. (September.)

The alga approaches *Sirogonium ventersicum* (Transeau, Tiffany, Taft and Li, " New species of Zygenmataceæ, " *Trans. Mic. Soc.*, Vol. LIII, No. 3, 1934, p. 229, Pl. XXII, Fig. 65) on account of the ellipsoid or rarely somewhat ovoid zygospores provided with a brown mesospore that is densely and irregularly verrucose, but differs from the same in the number of chromatophores and the dimensions of the vegetative cells and the zygospores. It further contrasts in the fructifying cells being inflated to a greater extent.

Sub-order Desmidioideæ.

Family Desmidiaceæ.

Genus *Closterium* Nitsch.

30. *Closterium acerosum* (Schrank) Ehrbg. Krieger in Rabenhorst's *Kryptogamen-Flora Von Deutschland, Österreich und Schweiz*, Band XIII, Abt. 1, Leipzig, 1935, p. 318, Taf. 24, Fig. 1 ; W. and G. S. West, "British Desmidiaceæ," *Roy. Soc. London*, 1904, Vol. I, p. 146, Pl. XVIII, Figs. 2–5 ; Ralfs, *The British Desmidieæ*, 1848, p. 164, Tab. XXVII, Fig. 2.

Lat. cell., 33–44 (–66) μ ; long. cell., 365–562 (–721) μ.

Habitat.—In paddy fields, Kurla. (September.)

31. *Closterium lanceolatum* Kütz. Ralfs, *op. cit.*, 1848, p. 164, Tab. XXVIII, Fig. 1 ; Krieger, *op. cit.*, 1935, p. 319, Taf. 24, Figs. 9–10 ; W. and G. S. West, *op. cit.*, 1904, Vol. I, p. 149, Pl. XVII, Figs. 9 and 10.

Lat. cell., 40–44 μ ; long. cell., 227–296 μ.

Habitat.—In a rain-pool, Dadar. (July.)

Genus *Cosmarium* Corda.

32. *Cosmarium Phaseolus* Breb. W. and G. S. West, *op. cit.*, 1904, Vol. 2, p. 158, Pl. LX, Figs. 12–14.

Forma.

Lat. cell., 21–22 μ ; long. cell., 22–24 μ ; lat. isthmus, 4–5 μ.

Habitat.—In a ditch near Vihar Lake. (December.)

This form comes very near forma *minor* Boldt., but is slightly bigger in dimensions.

33. *Cosmarium venustum* (Breb.) Arch. forma *minor* Wille. W. and G. S. West, *op. cit.*, 1904, Vol. 3, p. 10, Pl. LXVI, Fig. 4.

Lat. cell., 14·8–18 μ ; long. cell., 18·5–22·2 μ ; lat. isthmus, 6 μ.

Habitat.—In a pond near Mâhakâl Caves. (November.)

Genus *Staurastrum* Meyen.

34. *Staurastrum crenulatum* (Nag.) Delp. Krieger, "Die Desmidaceen der Deutschen Limnologischen Sunda-Expedition," in *Archiv für Hydrobiologie*, 1932, *Suppl.-Bd.*, XI, *Tropische Binnengewasser*, Band 3, p. 129–130 ; p. 196, Taf. XIX, Fig. 10.

Forma.

Long. cell., 29·6–33·3 μ ; lat. cell., 37–40·7 μ ; lat. isthmus, 7·4–9·5 μ.

Habitat.—In the aquarium, Wilson College, Bombay. (December.)

Order Siphonales.

Family Vaucheriaceæ.

Genus *Vaucheria* De Candolle.

35. *Vaucheria Thuretii* Woronin. Heering in Pascher's *Süsswasser-Flora Deutschlands, Österreichs und Der Schweiz*, 1921, Heft 7, p. 38, Fig. 69 ; Brown, "The Algal Family Vaucheriaceæ," *Trans. Amer. Mic. Soc.*, Vol. XLVIII, No. 1, 1929, p. 95, Pl. XVI, Fig. 18.

Diam. fil., 33–116 μ ; crass. oog., 182–200 μ ; long. oog., 232–250 μ ; lat. oosp., 166–182 μ ; long. oosp., 182–210 μ.

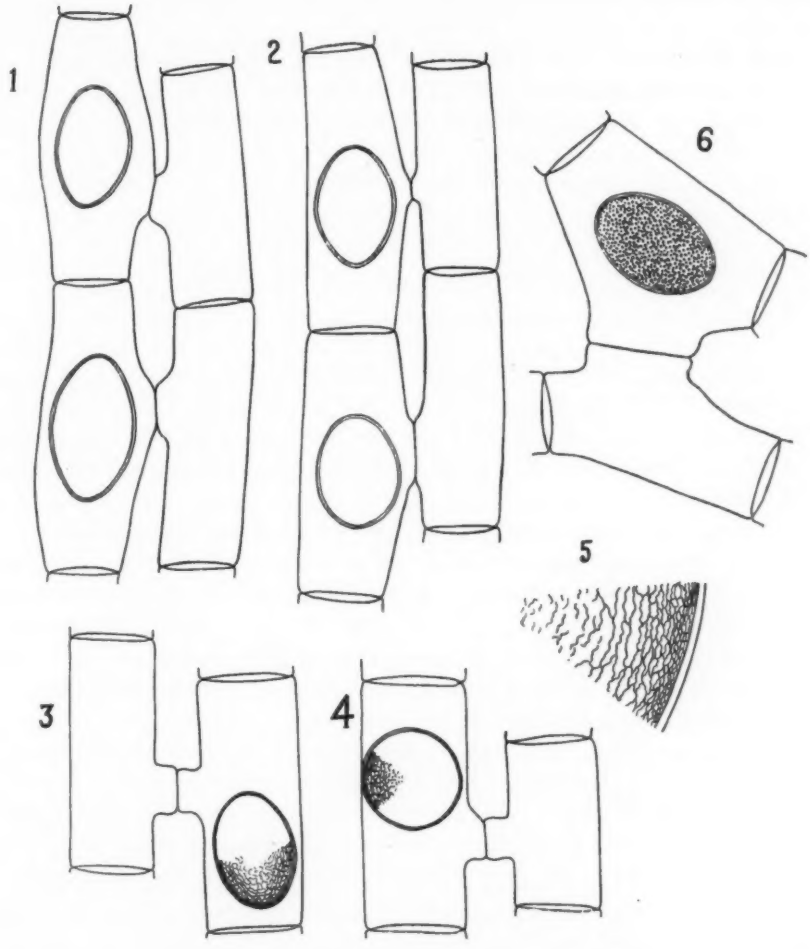
Habitat.—On the mud-flat near aerodrome, Santa Cruz. (January, V. N. Hate.)

36. *Vaucheria piloboloides* Thuret. Le Jolis, *Liste Alg. Mar. Cherbourg*, 1863, p. 65, Figs. 4–5 ; Brown, *op. cit.*, 1929, p. 101, Pl. XX, Figs. 40–41.

Diam. fil., 45–66 μ ; diam. oog., 83–160 μ.

Habitat.—On the sea-beaches, Colaba, Chowpathy and Juhu, Bombay. (October-February.)

Most of this work was carried out in the Botanical Laboratory of the Benares Hindu University and the author wishes to express his grateful thanks to Prof. Y. Bhâradwâja, M.Sc., Ph.D. (London), F.L.S., for his most valuable and generous help throughout the preparation of this paper. He is also thankful to Mr. C. Bhashyakarla Rao, M.Sc., for drawing the figures.



EXPLANATION OF THE TEXT-FIGURES.

Figs. 1 & 2.—Conjugating filaments and the zygospores of *Spirogyra gracilis* (Kützing) Czurda var. *magnum* var. nov.

Figs. 3 & 4.—Conjugating filaments and the zygospores of *Spirogyra bimorphis* Sp. Nov.

Fig. 5.———Surface-view of the mesospore of *Spirogyra bimorphis* Sp. Nov., highly magnified.

Fig. 6.———Conjugating filaments and the zygospore of *Sirogonium inflata* Sp. Nov.

Figs. 1 & 2 × 835. Figs. 3, 4 and 6 × 400. Fig. 5 × 1,725.

A REPORT ON THE GUINEA-WORM FOUND IN *VARANUS* SP., WITH A SHORT NOTE ON *DRACUNCULUS MEDINENSIS.*

By M. B. Mirza
and
M. A. Basir.
(*From the Zoological Laboratories, Muslim University, Aligarh, U. P., India.*)

Received December 21, 1936.

Three years ago, a specimen labelled Filaria from the muscles of *Varanus*, was handed over to one of us (Mirza) by Dr. D. R. Bhattacharya, Ph.D., D.Sc., Professor of Zoology, Allahabad University, and we take this opportunity to offer our sincere thanks to him for giving us this material for further investigation.

It was also brought to our notice that 60% *Varanus* were found infested with this worm, and sometimes as many as 10 to 15 worms were obtained from a single specimen. A large number of *Varanus* have been dissected in our laboratories also, but, so far we have not come across this parasite.

The worm was kept for a very long time in 70% alcohol and as it was not subjected to any kind of fixative, we had to encounter great difficulties in preparing it for a histological study.

Dracunculus is reported from various wild and domesticated mammals including the wolf, hunting leopard, pole cat, dog and horse. It has also been reported from snakes. In the United States of America and Canada, it has been reported from wild carnivores—silver-fox (*Vulpes fulva*), the raccoon (*Procyon lotor*), the mink (*Putorius vison*); and *Cnelydra serpentina*.

The worm from varanus measured 68 *cm. in length and* 1·3 *mm. in width.* A series of transverse sections were taken through the cephalic end and the internal structure has been compared with that of *Dracunculus medinensis*.

The anterior end tapers gradually and ends in a globular head which is not separated off from the body by a constriction as described for *Dracunculus houdemeri* Hsü, 1933. The mouth opening is oval and opens into a short muscular œsophagus with a tri-radiate lumen one arm of which is always directed ventrally. This is followed by a glandular œsophagus of considerable length. The three œsophageal glands—one dorsal and two sub-ventral—which are entirely separated from each other, are represented

here also. The glands are unicellular. The presence and absence of their nuclei depends upon the age of the worm. If a specimen of *D. medinensis* is extracted by wrapping it over a piece of wood, bit by bit every day—a process which lasts for well over three weeks—the nuclei of the glands degenerate and are no more to be traced as the glands give up the function of secretion and become reservoirs for the storage of reserve material. *The nuclei of all the three glands could be noticed in specimens which are obtained by a surgical operation while the worms are still young* and lie entirely under the sub-cutaneous tissues.

Hsü, in his observations on *D. houdemeri*, *D. medinensis*, and *D. globocephalus*, mentions : " The opening of dorsal gland was not found by Mirza in *D. medinensis*" (*Zeit. f. Parasit.*, Bd. 6, Ht. 1, 1933, pp. 114), whereas Mirza has clearly shown the opening of the dorsal gland into the muscular œsophagus (*Zeit. f. Parasit.*, Bd. 2, Ht. 2, 1929, Fig. 14) and on page 143, it is mentioned : " In kurzer Entfernung vom Vorderende mündet lateral in den Ösophagus der Ausführungsgang der grossen Drüse ein (Abb. 14, kurz hinter a)." Further on page 147, it is stated : " Am Vorderende steht die grosse Drüse durch einen engen, aber stets deutlich erkennbaren Kanal mit dem Ösophagus in Verbindung (Abb. 14)." It is true that Mirza could not trace the openings of the two sub-ventral glands into the œsophagus.

In two separate series of cross-sections of *D. medinensis*, which we have prepared recently, the nuclei of the dorsal and the two sub-ventral glands have been noticed by us. In the first series of sections, the nucleus of the dorsal gland was absent while the nuclei of the two sub-ventral glands could be seen (Fig. 1). In the second series, all the three nuclei could be seen but the nucleus of the dorsal gland was undergoing degeneration (Fig. 2). These observations reveal the fact that *the three nuclei of the three glands could easily be traced in a comparatively young specimen.*

As regards the structure of the nucleus of the dorsal gland, we confirm Hsü's observations. It is very long and irregular in size and differs in different regions. It is the nucleus of the dorsal gland that degenerates first and then disappears. From this, it is evident that the gland does not perform the function of secretion. Our series of cross-sections through worms of different ages lead us to the conclusion that as the nucleus of the dorsal gland starts to degenerate, the gland gives up the function of secretion and becomes a reservoir of reserve material.

Hsü further mentions : " The contents of the two sub-ventral glands of *D. houdemeri* appeared similar to what I describe for the dorsal gland. These two sub-ventral glands also showed the same kind of staining reaction as the

dorsal gland. In *D. globocephalus*, however, the two subventral glands stained differently as compared with the dorsal gland ; that is, in a hematoxylin-eosin preparation the contents of the dorsal gland took a dark blue color, whereas those of the two sub-ventral glands stained orange red." Our observations with the same staining in the two series of sections are that in one series of a comparatively young specimen a part of the contents of the dorsal gland, of *D. medinensis*, took a blue colour and a part orange red while those of the two sub-ventral glands stained orange red only. In the second series, the contents of all the three glands stained orange red. From our observations and those of Hsü's, we infer that the contents of all the three glands of an old specimen are the same hence we are inclined to believe that in an advanced age all the three glands become reservoirs for reserve material.

FIG. 1*b*.

Mackin's specimen of *D. globocephalus* was a young worm as mentioned by Hsü, hence the opening of the outlet tubules of the two sub-ventral glands could be traced, as well as the lumen of the œsophagus which is obliterated in certain parts in old specimens. It is a pity that Hsü's specimen of *D. houdemeri* was damaged at this particular region otherwise he too, perhaps, would have failed to trace the outlet tubules of the two sub-ventral glands of *D. houdemeri*. Perhaps, in certain specimens the two sub-ventral glands open in the region of the obliterated lumen of the œsophagus or in other words, into the dorsal gland hence the opening of the outlet tubules of the sub-ventral glands into the œsophagus is no more to be seen. Hsü has given a very instructive diagrammatic reconstruction of the œsophagus (Fig. 38), but *it is reconstructed not from worms of the same species but from worms of two different species*. We cannot draw conclusions by combining the characters of two different species in one.

We do not attach importance to the distribution of the nuclei of ordinary and marginal muscle fibres, especially those of the glandular œsophagus, as these also degenerate with the increasing age of the worm. Their number could be determined in young specimens only, and would be true for specimens of a particular age. The regions of the deformation of the œsophagus differ in different specimens, consequently the number and position of the nuclei also varies.

The position and the nature of the nerve-ring in *Dracunculus* from *Varanus* is just the same as found in *D. medinensis*. The problematic body which has been observed by former authors in *D. medinensis* and described by Fedschenko (1870) as, " ein äusserst interessantes Gebilde, wahrscheinlich von Drüsenart " ; by Leuckart as, " der schon mehrfach erwähnte problematische Körper, den ich als Bauchganglion deute " ; by zur Strassen as, " der-allerdings enorme-Kern einer dorsalen Schlund-drüse " ; and by Mirza as, " eine tripolare Nervenzelle," is also present in the worm in question (Fig. 3, *a*) and is of the same structure as mentioned by Mirza (1929) in case of *D. medinensis* (Fig. 3, *b*). After a careful study, *we hold that this body is nothing else but a tripolar nerve-cell of an enormous size.*

The posterior end of the worm is characteristic of *D. medinensis*. The rectum is short and ends blindly. As the worm was not in a good condition at all, the rectal glands could not be traced but these have been seen in *D. medinensis*. The vulva is also absent. The embryos of this specimen possess identically the same characters and size as those of a badly fixed specimen of *D. medinensis*.

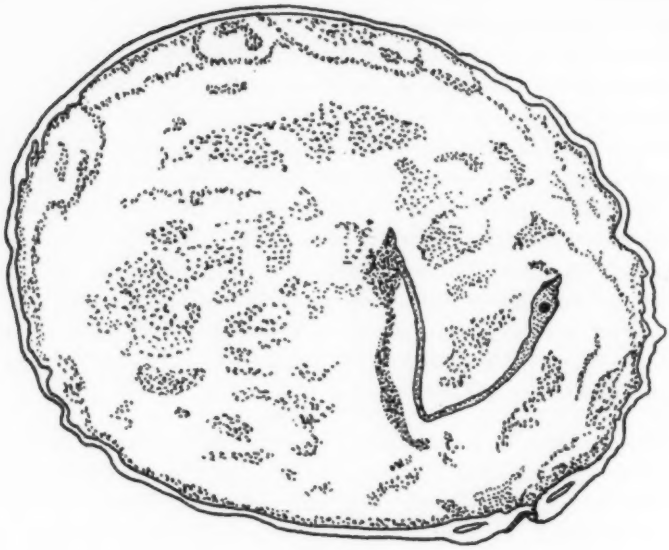


FIG. 2*b*.

We could not study the number and disposition of the head papillæ of the worm hence these could not be discussed in the present paper. As the infection is reported to be very heavy, we shall discuss this and various other points which we are forced to leave out at present, after procuring well-preserved material.

Varanus is studied by B.Sc. class students of most of the Indian Universities as a type of Reptilia. Perhaps, the specimens obtained by us so far were not from the same locality as those obtained by the Zoology Department of the Allahabad University. Prof. L. P. Mathur of St. John's College, Agra, also reported to one of us (Mirza) that he too came across a number of *Dracunculus* infesting the sub-cutaneous tissue and the body cavity of *Varanus*, but unfortunately, these were not preserved. If the material could be obtained in such a quantity as has been told to us then much more light could be thrown on the subject. We are looking forward to such an opportunity when a more detailed study could be undertaken. It is strange that our observations be based on a single badly fixed specimen when there seems to be no scarcity of material, but such are the conditions under which an Indian researcher in India has got to work. In our opinion, *Dracontiasis* must be prevalent in that area where the infested *Varanus* were procured. *Varanus* relish and live upon insects as well as crustaceans. They are fond

of water and feed, surely, on cyclops harbouring the *Dracunculus* larvæ. The intensity of infection in these reptilian hosts is a definite proof in support of our view.

Conclusion.—Turkhud (1920) described *Dracunculus* from *Naia tripudians* and considered it to be *D. medinensis.* Hsü is inclined to take it for *D. houdemeri* considering the size of the embryos of Turkhud's specimen. Turkhud's material is not available for study but after a thorough study of the *Dracunculus*-material in our possession and knowing the conditions prevalent in India, we are inclined to think that it could not have been anything else but the same worm as has been found in *Varanus.*

Chitwood (1933) mentions ten cases of *D. medinensis* reported from man in the United States of America, out of which " four are certainly or possibly of foreign origin and six are not cases of *D. medinensis.*" He continues, " It would appear that our native wild mammals harbour this highly important parasite, *Dracunculus medinensis,* which is most widely known from man in the Old World. It may be that this is a physiologic strain or variety which is not adapted to man or that living conditions here account for the fact that up to the present time it has not appeared in naturally acquired infestation as a parasite of man on this continent....."

Chitwood holds that the *Dracunculus* occurring in the fox, raccoon and mink in Nebraska, Iowa, New York, Pennsylvania (?) and Ontario, is morphologically identical with *D. medinensis,* and probably is *D. medinensis.*

We are not in a position to say that the *Dracunculus* parasitic in animals is a physiologic strain or variety which is not adapted to man, but after studying the morphology of the specimen obtained from *Varanus,* we are inclined to believe that the *Dracunculus* of human beings is a variety which is adapted to animals also, and *we hold that the guinea-worm obtained from* Varanus *sp. is* Dracunculus medinensis.

Summary.

Dracunculus is recorded for the first time as a parasite of *Varanus.*

The structure of the worm has been compared with that of *Dracunculus medinensis* and it is concluded that it is the same worm which parasitizes the human beings.

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EXPLANATION OF FIGURES.

FIG. 1.—*Dracunculus medinensis.* Cross-section through the glandular œsophagus, showing the nuclei of the two sub-ventral glands. (*a*) Photomicrograph, (*b*) Diagram.

FIG. 2.—*Dracunculus medinensis.* Cross-section through the glandular œsophagus in the posterior-most region of the two sub-ventral glands, showing the nucleus of the dorsal gland undergoing degeneration. (*a*) Photomicrograph, (*b*) Diagram.

FIG. 3.—Cross-section through the tripolar nerve-cell and the glandular œsophagus at slightly different levels of (*a*) *Dracunculus* from *Varanus*, (*b*) *Dracunculus medinensis.*

1733-36—Printed at the Bangalore Press, Bengalore City, by G Srinivasa Rao, Suparintendent, and Published by The Indian Academy of Sciences, Bangalore.

FIG. 1*a*.

FIG. 2*a*.

The sections have been projected direct on the printing paper through Abbe prism. A new and simple method for taking photomicrographs devised by one of us (M. A. Basir). The paper will be published elsewhere.

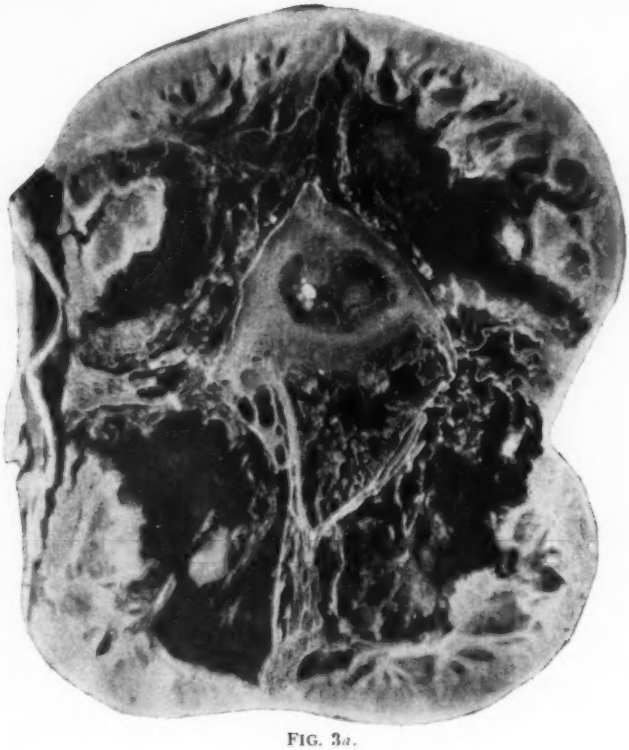

FIG. 3*a*.

FIG. 3*b*.

STUDIES ON THE TREMATODE PARASITES OF BIRDS.

Part I. Value of Different Characters in the Classification of Avian Trematodes.

By Makund Behari Lal.

(From the Department of Zoology, Lucknow University, Lucknow, India.)

Received January 4, 1937.

[Communicated by Dr. G. S. Thapar, M.Sc., Ph.D. (London).]

While working on the trematode parasites of birds the author has been confronted with difficulties in assigning a proper systematic position to some of his newly discovered forms. A reference to the literature on the subject also shows divergent views on several points. It has, therefore, been thought desirable to survey in the present communication the factors governing the classification of the group; and to remove as far as possible the existing confusion regarding the merits of different characters.

The author is deeply indebted to Dr. G. S. Thapar for his valuable criticisms and suggestions and also for the free access to his vast library without which a work of this nature would not have been possible.

The classification of Avian trematodes presents many difficulties. This is due to the fact that adequate diagnosis of a number of genera and species is not available and where such descriptions are available, the accounts are incomplete and illustrations few. It would not be possible at present, however, to bring the classification on a sound natural basis but the study of some new forms from India and a review of the older ones necessitates the revision of their classification.

The general tendency of the various workers indicates that one or the other character has been regarded by them of preferential value in the classification of digenetic trematodes and thus the diagnosis of the families, genera and species is based on such characters. The characters which have been emphasised for this purpose from time to time are the excretory system, suckers, intestine and pharynx, oral collar, gonads, cirrus-sac, receptaculum seminis, genital pore, uterine coils and vitellaria.

Some of these characters can be traced down to the larval stages. A few of them are modified or suppressed during development, but there are some characters that are conspicuous by their constant presence both in

the larvæ and the adults and such characters should, in the writer's opinion, form the primary basis for the classification of the group. These characters are discussed here to show their relative importance in the classification of trematodes.

Excretory System.

Much stress has been laid on the study of the excretory system of trematodes and there is no doubt that this character has a vital importance in the classification. It has been considered under different heads, chiefly the type of the excretory bladder, position of the excretory pore and the general disposition of the entire system. Although the shape of the bladder is a constant feature, some workers have given it a specific value, *e.g.*, in the genus *Ganeo*. It should, however, be considered a character of generic importance. The position of the excretory pore was regarded to be an important distinction between the sub-families Opisthorchiinæ and Metorchiinæ but Morgan (1927) does not consider it to have more than a generic importance. In *Halipegus* and some other genera the position of the excretory pore has been regarded only of a specific value. But the primitive condition of the excretory system as seen in cercaria represents the type in the adult stage and since this system of organs is least modified during development, it should be regarded of a family importance in the classification. Faust (1930) advocates this view when he says, "Arrangement of excretory system which has been found to be identical in the cercarial larvæ of three human Schistosome species is an admirable illustration of the fact that the excretory system undergoes little change owing to the adaptations of the parasitism and is of value in classification." Brown (1927) on his studies on the life-history of *Crepidostomum* says, "One of the most important systems and one which is invaluable in a life-history study is the excretory system". The number of flame cells and their multiplication is fixed for the various families and gives us the true indication of the relationships of the forms possessing them. Sewell (1922), as a result of his work on Indian cercariæ, very strongly argues for regarding the excretory system as a basis for classification. Although it is difficult to follow the exact details of the branching in the adult trematodes, the main arrangement of the system is sufficiently clear to give us a clue to the true position of the individual.

Suckers.

The position of suckers has formed an important feature in the classification of digenetic trematodes. Thus in the Gasterostomata the oral sucker occupies a mid-ventral position. The presence and absence of an oral sucker is considered of generic importance in the families Aspidogastridæ and

Psilostomidæ. The ventral sucker which normally lies on the ventral side comes to lie at the posterior end in Paramphistomoidea. The presence and absence of ventral sucker distinguishes the genera in the families Spirorchidæ and Cyclocœlidæ.

The relative size of the oral and ventral suckers is regarded of specific importance by Holl (1928) in *Gorgoderina*. The family Heterophyidæ is distinguished by the presence of a genital sucker. Apart from these various considerations in the adult trematodes, the oral and ventral suckers are of constant occurrence in the cercariæ and form a useful character for their systematic identification. The position of the ventral sucker as seen in the Paramphistomoidea is traceable to the amphistome cercariæ and should form an independent distinguishing character for the group. Cercariæ with a ventral sucker generally develop into the distomate forms while those without it develop into the Monostomes. Thus it is evident that although the position of the suckers in the Gasterostomata and Paramphistomoidea may be very important, the presence of an oral or ventral sucker is not of such fundamental importance. Invariably it cannot be regarded as a family character because in the same family we come across forms which either possess or lack one or even both the suckers. For instance, forms under Cyclocœlidæ show interesting gradations as regards the suckers. This character should, therefore, be regarded of a generic importance. Although a genital sucker is considered of family importance in Heterophyidæ, Dawes (1936) regards it only as a character of specific importance in *Paramphistomum*. The genital sucker, therefore, could only be a good basis for the creation of a genus and it should not be assigned a family importance as has been done in some cases.

Intestine and Pharynx.

Here we have a number of characters, such as

(*a*) Cæcal union at the posterior end.
(*b*) Cæcal diverticula.
(*c*) Length of intestinal cæca.
(*d*) Anus.
(*e*) Pharynx.

(*a*) This character is considered of generic importance in forms like *Progonus*, *Opecoelus*, etc., while it has been considered an important character in the diagnosis of the family Cyclocœlidæ and Coitocæcidæ. The cæcal union has been traced in one case to the monostome cercaria, *Cercaria kobayashii* and there seems to be no doubt in regarding this character of at least a generic importance.

(*b*) Joyeux and Baer (1927) regard the presence or absence of cæcal diverticula of generic value in the family Cyclocœlidæ while Witenberg (1926) took it to represent a sub-family character. Cameron (1934) distinguished the sub-families Fasciolinæ and Fasciolopsinæ from each other on the basis of this character and Lal (1936) considered the advisability of retaining the sub-families Typhlocœlinæ and Cyclocœlinæ owing to the presence or absence of cæcal diverticula. The larval history of forms points out that the cæcal diverticula make their appearance soon after the metacercaria stage and continue to extend till they have attained their maximum size in the adult. Where the beginning is not made soon after the metacercaria stage the adults do not show any cæcal diverticula. These facts further show the systematic importance of this character and the author is inclined to regard it as a character of sub-family importance.

(*c*) The extent of the intestinal cæca is a character which affords interesting material for discussion. Here we come across two divergent views from the same school. Mehra (1935) considers this character to have a sub-family importance in the family Lecithodendriidæ although his pupils earlier considered this character to have only a specific value in some members of the same family. Thus, Srivastava (1933) separates the species in the genus *Ganeo* and Chatterji (1933) differentiates between the species of *Astiotrema* on the basis of this character. In the genus *Haplorchis* (Heterophyidæ) also this character has a specific value. Dawes (1936) regards the relative backward extension of intestinal cæca as a specific character in *Gastrothylax*. The generic significance of this character is indicated by Mehra and Negi (1926) in the creation of the genus *Tremiorchis* (syn. *Centrovitus* Bhalerao, 1926). Although much light is not thrown on this point by ontogeny, this character appears to be subject to variations by pressure and unless there are extreme and constant differences it should not have more than a specific value or at best a generic importance.

(*d*) The presence of anus has been regarded of generic importance in *Balfouriella*, *Chaunocephalus*, *Haplocladus*, *Opegaster*, etc. This connection of the alimentary tract with the exterior may be direct or through the excretory bladder; there may be a single opening or there may be separate pores. Ozaki considered the presence or absence of an anus as a notable and important character, although Stunkard (1931) does not consider it a character of great taxonomic importance. An anal opening has been observed in the metacercaria of *Pharyngora bacillaris* and it is, therefore, suggested to regard this character of generic value.

(*e*) The absence of pharynx is regarded only of sub-generic importance by Cameron (1934) who maintains the two sub-genera *Watsonius* and

Pseudodiscus, the former lacking a pharynx and the latter possessing it. The character has a generic importance in *Apharyngostrigea* and a family importance in Notocotylidæ, Schistosomidæ and other blood-flukes. A pharynx is mostly present in the cercaria except in a few furcocercous forms or in some monostome cercariæ belonging to Notocotylidæ. On tracing the development of such forms it has been observed that such apharyngeal cercariæ always give rise to adults which lack a pharynx. An exceptional case is reported by Price (1929) who has referred to the characters of *Bilharziella yokogawai* and quotes that a pharyngeal cercaria was observed in this blood-fluke by Oiso (1927). This is rather an interesting case and may on confirmation in some other cases show a possible transition from pharyngeal to apharyngeal forms. In view of these observations the author considers that the absence of pharynx should be regarded as a generic character.

Oral Collar.

The circum-oral collar may bear spines or may be smooth. The former is a distinguishing character of constant occurrence in the family Echinostomidæ both in the cercaria and the adult. It may, therefore, be regarded of family importance. The arrangement of spines on the circum-oral collar varies in the Echinostome cercariæ; some forms, *e.g.*, *Cercaria oscillatoria* show a single row, while in others, *e.g.*, *Cercaria limbifera* a double row of spines is present. Although much data is not available in this connection it may be pointed out that the arrangement of spines on the collar alone would not be sufficient to justify a distinction of more than a specific value. A circum-oral collar without spines is not considered even of sub-family importance. Nicoll (1909) separates the genera *Stephanophiala* and *Crepidostomum* from the sub-family Bunoderinæ although all of them possess a circumoral collar.

Gonads.

The position of the ovary in relation to testes has been regarded of a family status in Plagiorchidæ, Dicrocœliidæ, and Opisthorchiidæ. But this character forms a sub-family basis in the classification of Pronocephalidæ. Cameron (1934) also, in his synopsis of the family Heterophyidæ, mentions it of a sub-family importance in Haplorchinæ and Adleriellinæ. Some workers have greatly discounted its value and regarded it only of specific nature, *e.g.*, Pande (1935) in *Lecithodendrium* and Khan (1935) in *Cyclocœlum*. Even Joyeux and Baer (1927) consider the pretesticular and intertesticular position of the ovary as a specific character in the family Cyclocœlidæ. Pratt (1902), Lühe (1909) and Ward (1918) have all laid emphasis on the relative position of ovary and testes. Stunkard regards the relative position of

ovary and testes as a generic character in the family Spirorchidæ. It is, however, difficult to trace this character to the larval stages and we have to confine ourselves to the morphological data. Although it is not always advisable to base one's classification on the presence of a single character, the relative position of the gonads may be taken of generic importance. The connubial and tandem positions of the testes alone have formed a character of generic value in several cases. But Srivastava (1933) regards the position of testes in *Ganeo* as indicating only a specific character. Similarly Sinha (1932) considers the tandem or diagonal position of testes in *Cephalogonimus* to be a specific character. As opposed to this Cameron (1934) considers the connubial, tandem or oblique positions of testes of generic importance in Heterophyidæ. In case of *Paramphistomum* he retains, however, the sub-generic value of this character in distinguishing the sub-genera *Buxifrons* from *Explanatum* and *Carmyerius* from *Fischœderius*. Dawes (1936) in his revision of the genera *Paramphistomum* and *Gastrothylax* considers the tandem or connubial position of testes as merely a specific character.

Cirrus Sac.

This structure has been considered as a character of distinction between the families Plagiorchidæ and Opisthorchiidæ but it is regarded only of generic value in the families Lecithodendriidæ, Aspidogastridæ and Spirorchidæ. The sub-family importance of this character is emphasised in Acanthostomidæ and Strigeidæ. The appearance of a cirrus-sac in some Agamodistomes, *e.g.*, that of *Cercaria thalia* proves its value in systematic diagnosis and this character, therefore, appears to be of a sub-family importance.

Receptaculum Seminis.

The absence of receptaculum seminis has been regarded of a family importance in distinguishing Psilostomidæ from Allocreadiidæ. It also forms a character of family importance for Heterophyidæ. But this character has been put to a great discount by Khan (1935) in *Cyclocœlum* and Dietz (1909) in Echinostomidæ. Similarly the absence of receptaculum seminis is regarded as a specific difference in the species of *Ityogonimus*. Its generic value is seen in the family Spirorchidæ and in the genus *Ganada* (Plagiorchidæ). The fact that a receptaculum seminis has always been demonstrated in the trematode possessing it, obtained at any season of the year, shows its permanent nature. Its development in some of the Agamodistomes further confirms this. This character should, therefore, have a generic and in some cases even a sub-family value in the classification.

Genital Pore.

The position of the genital pore has been considered to be a family character in Cephalogonimidæ. This has been taken as a distinguishing feature of the sub-families of Notocotylidæ, Acanthostomidæ and Plagiorchidæ. It has invariably been assigned a generic importance in distinguishing *Lebouria* from *Podocotyl*, *Parabascus* from *Phaneropsolus*, and *Protenes* from *Cercorchis*. The genus *Lecithopyge* has been created on the position of the genital pore and Perkin (1928) remarks that the position of genital aperture is a character of generic significance throughout the sub-family Telorchinæ. Lal (1935) recently assigned a generic importance to this character in some members of the sub-family Notocotylinæ. Yamaguti (1936) in his recent paper creates a new genus *Lepocreadioides* on the position of the genital pore and in fact distinguishes it from the closely related genus *Lepocreadium* on the basis of this character alone. It is, therefore, clear that the position of the genital pore, if constant, should be sufficient to distinguish a genus.

Uterine Coils.

The disposition of uterine coils was regarded to be of a sub-family importance in the family Plagiorchidæ. It was also taken to distinguish the sub-families Opisthorchiinæ and Metorchiinæ but Morgan (1927) regards it only of generic importance. It is, however, regarded as a character of distinction between the families Cephalogonimidæ and Plagiorchidæ. The peculiar arrangement of the uterine coils in the family Telorchidæ sharply distinguishes it from Plagiorchidæ. The uterus has a limited distribution in the family Allocreadiidæ. Although these facts show the importance of this character as a systematic unit yet the position of the uterus alone should not have much value as this is subject to variation according to the number of eggs produced. The uterus has, however, a definite relation to the gonads in most cases and the relative position of the uterine coils and gonads could be considered as satisfactory basis for the distinction of genera in some cases.

Vitellaria.

The nature and extent of vitelline glands has been a much disputed point in the taxonomy of trematodes and should be considered with great reservations in the classification. The position of vitellaria has undoubtedly formed a constant feature in some families, *e.g.*, Philophthalmidæ, Dicrocœliidæ, etc., but generally there is a great variation in their position and arrangement. Although the family Echinostomidæ and Psilostomidæ were supposed to have a constant position of their vitellaria, recent

discoveries have revealed that their position is so variable in these families that in some cases this character has merely a specific value. Also in the genus *Ityogonimus* this character has a specific importance. Perkins (1928), however, finds that the position and extent of vitellaria has a generic significance in Telorchinæ. The genus *Cercolecithos* is distinguished from other Telorchinæ specially from *Cercorchis* in the extent of vitelline glands behind and over the testes. Sandground (1934) thinks that this character is invested with primary value in the classification of Strigeidæ. In view of these varied opinions and also as a result of the author's own observations it would be better to consider this character on the individual merits of the forms.

Besides the characters mentioned above, the sexuality in trematodes is also important and the family Schistosomidæ is distinguished from all other digenetic trematodes in having distinct males and females. The nature of eggs, which so often helps in the clinical diagnosis of human helminths, is of great value in the diagnosis of the genera in the sub-family Himasthlinæ. The genera *Lebouria* and *Helicometra* of the family Allocreadiidæ are differentiated by the presence or absence of egg-filament.

Host-Specificity.

Before dealing with the systematic work on the Avian trematodes reported from India, it would be worth while to discuss the host-parasite relationship and structural variations in trematodes.

The common belief, that the larvæ of helminths if they enter an abnormal host may wander about aimlessly and ultimately perish, is based on the host-specificity hypothesis of older helminthologists. It has now been established more than once that a single species of trematode may inhabit two or more hosts. Instances are numerous, that of *Ommatobrephus folium* parasitic in *Tropidonotus piscator, Zeminis natrix, Naja tripudians,* etc. *Xenopharynx solus* has also been recorded from two hosts: Nicoll (1912) described it from *Naja tripudians* and Khalil (1923) described the same species from *Naja bungarus*. Such instances have multiplied in recent years. Mention may also be made of the important work of similar nature on the non-specificity of hosts in cestodes by Meggitt (1934). It is true that the widely different hosts like Fish, Bird or Mammal raise a doubt as to the close relationship of their parasites but this point has not much significance. A large number of species of *Opisthorchis* have been reported from Fish, Birds and Mammals yet they all belong to one genus. Nicoll (1909) was confronted with similar difficulty when he described the genus *Stephanophiala* from the common trout. In discussing the systematic position he

compared *Crepidostomum metœcus* with *Crepidostomum laureatum*, the former found in bats and the latter in fish. Nicoll, however, agreed with Braun that too great weight need not be attached to this as the larval stages of both may be passed in insect larvæ forming food common to bat and fish.

Structural Variations.

Vevers (1923) on his studies of the genus *Paragonimus* confirms the observations of Ward and Hirsch (1915) that in this genus there is an extreme variability in the shape and size of the body and internal organs on which specific differences were previously based; and found that the cuticular spines alone could differentiate the species from each other. Similarly variations may be seen in other trematodes and allowance should always be made of the individual variations in assigning them to their true systematic position.

Conclusions.

From the foregoing discussion it would appear that the classification of digenetic trematodes has been based on their morphological characters. Of these only those characters which are present both in the larvæ as well as in adult should be considered of primary importance in the classification. Other characters whose values have already been discussed may be considered individually on their own merits.

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*CHABERTIA RISHATI** N.SP.—A NEW NEMATODE PARASITE OF CAMEL.

By S. A. Akhtar,

Prof. of Biology, Faculty of Medicine, Kabul, Afghanistan.

Received December 23, 1936.

(Communicated by Dr. M. B. Mirza, D.Sc.)

Three pairs of the genus *Chabertia* Railliet and Henry, 1909, were obtained from the large intestine of a camel. Anterior end is obliquely truncate. Mouth, facing antero-ventrally, is with two very small leaf-crowns anterior of which is with very small elements, while the other one is without them. A faint transverse ventral cervical groove is present preceded by a slight indication of a cephalic vesicle. Buccal capsule is sub-globular and œsophagus with lamellæ.

Male.—Buccal capsule contains a plate of knobs, with small terminal spines, at its depth. Accessory piece as well as pre-bursal papillæ are present. The spicules are slender and equal. The bursa is the same as in Oesophagostomum.

Measurements.—

Length of the longest worm	17·775 mm.
Thickness of the worm	0·556 ,,
Length of buccal capsule	0·304 ,,
Width of buccal capsule, antero-dorso-ventrally	0·292 ,,
Cervical groove from anterior end	0·618 ,,
Length of œsophagus	1·215 ,,
Length of spicules	1·575 ,,
Length of accessory piece	0·126 ,,

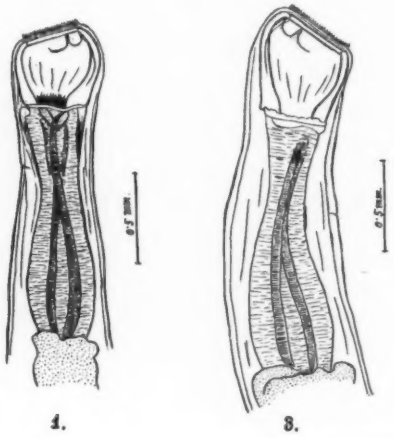
Female.—There is no plate of knobs with small terminal spines at the depth of buccal capsule. Vulva is prominent, near and anterior to anus. Tail ending in a spine and curved dorsally.

Measurements.—

Length of the longest worm	23·411 mm.
Thickness of the worm	0·867 ,,
Length of buccal capsule	0·450 ,,

* The writer feels pleasure in naming this species after Dr. Hasan Rishat Beg, President, Faculty of Medicine, Kabul.

Width of buccal capsule antero-dorso-ventrally	0·315 mm.
Cervical groove from anterior end	1·012 ,,
Length of œsophagus	1·348 ,,
Vulva from anus	0·281 ,,
Size of eggs .. .. .. 0·051 ×	0·041 ,,
Length of tail	0·292 ,,



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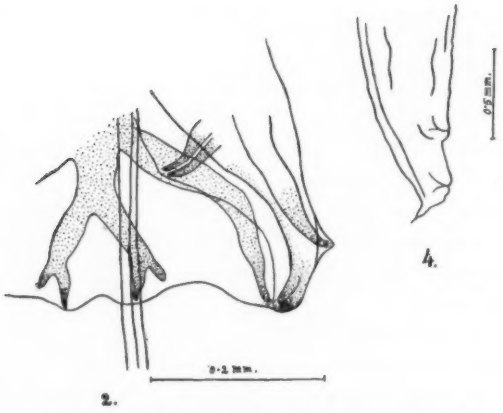

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Fig. 1.—Anterior extremity of male, lateral view.
Fig. 2.—Bursa, ventral view.
Fig. 3.—Anterior extremity of female, lateral view.
Fig. 4.—Posterior extremity of female, lateral view.

Only one species of the genus *Chabertia* has been so far described from goat, sheep and cattle. The species discovered by the writer closely resembles *C. ovina* (Gmelin, 1790) in having its mouth directed antero-ventrally and a faint ventral cervical groove ; but it differs in spite of different measurements, which show the worm to be stouter than *C. ovina*, in having small elements on only one and the anterior leaf-crown, in having œsophagus with lamellæ and the male having a plate of knobs with small terminal spines in the depth of buccal capsule. These characters are enough to create a new species and in this connection it is suggested that the presence or absence of lamellæ in the œsophagus may be added to the generic characters of *Chabertia*.

The writer expresses his sincere thanks to Dr. M. B. Mirza, Director, Zoological Laboratories, Muslim University, Aligarh, for kindly reading over the above account of the species.

Type-specimens have been deposited in the museum of the above named laboratories at Aligarh under No. 938.

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AN ANALYSIS OF CERTAIN CRITICISMS AGAINST THE EXISTENCE OF THE GOLGI APPARATUS.

By M. K. Subramaniam, M.A., D.Sc.

(From the University Zoological Research Laboratory, Madras.)

Received January 12, 1937.

(Communicated by Professor R. Gopala Aiyar.)

A study of the existing literature on the Golgi apparatus reveals that several misconceptions exist regarding its position, structure, shape and morphology. It seems that much of the confusion can be avoided if a dispassionate study is made without in any way importing personal inclinations into the subject. Protozoa forms only one of the fields, and the tendency to treat the shape, structure and function of the Golgi apparatus in any particular type or types of cells as distinct, separate and unrelated, has contributed in no small measure to the existing confusion. To cite an instance, oogenesis workers consider the structure, shape and function of the Golgi apparatus as peculiar and limited to the phenomena of oogenesis alone and at the same time assert that the Golgi apparatus does not arise *de novo*. But Bowen's (1923) comparison of the secretion of the acrosome by the Golgi apparatus to the secretion of various substances in gland cells is accepted without reservation : in many cases not fully cognizant of the implications of such an acceptance. The sperm has been shown to carry no Golgi apparatus into the egg in the majority of cases and since the Golgi apparatus does not arise *de novo* the Golgi apparatus of the eggs gives rise to the Golgi apparatus of all the cells which are derived from the zygote. This fact alone will show how it cannot be said that the shape, structure and function of the Golgi apparatus are peculiar to any particular cell or cells. There should be—and there is—some fundamental plan in the Golgi apparatus common to all cells and the different patterns in different cells if analysed should resolve—and do resolve—into numberless variations of a single procedure. Most of the cytologists do not seem to have realized the truth of this. Even in 1936 we find Dr. Gatenby emphasizing the existence of the Golgi apparatus in cells. The evidence for such an assertion is that the Golgi apparatus even in mammalian cells when centrifuged at high velocities is displaced from its normal position. As bodies of different specific gravities will move to different poles, actually the centrifuge

experiments do not prove the existence of the Golgi apparatus. On the other hand, if the existence of the Golgi apparatus is accepted it disproves Cowdry's (1925) conclusion that "as in mammals the fundamental material is not easily displaced by centrifugation, it is safe to assume that it is probably of about the same specific gravity as the remainder of the cytoplasm" (p. 341). As such repeated assertions about the existence of the Golgi apparatus have been and are still a bar to the progress of the study of the shape, structure and function of the Golgi apparatus I shall analyse here some of the fundamental causes for such a state of affairs. These are :—1. The laying of too much importance on technique forgetting that the apparatus has a morphology, position in the cell, size, constitution, function and behaviour. 2. Ignoring the fact that the apparatus is a living cell inclusion and *not merely a substance.* 3. Too much insistence on personal opinion without due regard to opinions held by others. 4. The argument of some that the apparatus has no existence in the living cell. To substantiate my contention I shall quote a relevant passage from a recent review on Protozoa (Hill, 1933). "Brown considers that in attempting to recognize Golgi material in the Protozoa the following characteristics should be emphasized :—(1) Consistent, not merely occasional impregnation by osmic methods ; (2) Resistance to the usual methods of bleaching after osmication ; (3) Consistent impregnation by silver methods ; (4) Occurrence in Protozoa generally and not merely in certain species ; (5) General similarity in form in different Protozoa" (p. 232). This leaves out its position in the cell, structure, vegetative division, size, behaviour during cell division and function. The tests given in the above quotation deal only with the technique for demonstration of a particular material in the cell and does not at all give any criteria for recognizing the body as a living inclusion—and a living inclusion has to assimilate, grow, divide and be passed on from cell generation to cell generation.

Coming to the third point namely, how personal opinions based on work in some particular field have marred a proper appreciation of the results in other fields I shall begin with Cowdry's review (1925) on the Golgi apparatus. The much talked of disagreement between workers does not seem to be so much due to conflicting results as to personal opinions, lack of clear knowledge of previous literature or a mistaken interpretation of other workers' results. The idea underlying this communication being to clarify some of the issues, I shall show from Cowdry's review how personal bias has tended to increase the existing confusion.

Cowdry mentions that Gatenby may be a little premature in his statement that the Golgi apparatus or its representative is present in every cell

of vertebrates and invertebrates and that the first exceptions to suggest themselves are the non-nucleated red blood cells. The nucleus has been considered a fundamental attribute of a cell and the very fact that a nucleus is not present removes the red blood corpuscles from the category of normal cells. He himself suggests that if we search through adult tissues, we will find the Golgi apparatus present in one form or another in each and every cell, except in those which are dead or dying. If that is so, one wonders how the red blood corpuscles form an exception.

Cowdry accepts the reversal in position of the Golgi apparatus, its behaviour as a unit in developmental cycle, its occurrence in invertebrates as isolated rods and granules, breaking up of the network during dictyokinesis and its manifestation as an integrating and unifying principle in vital processes, but refuses to admit Gatenby's declaration that the Golgi bodies are able to grow, assimilate and divide in the cytpolasm. After considering the apparatus all along as a ' material which behaves as a unit in developmental cycle ', he makes the suggestion: " This idea of the independence or individuality of chemical substances, expressed in relation to the Golgi apparatus, in a system which is in itself a co-ordinate and indivisible whole, may not make a strong appeal to those of us who are concerned chiefly with the somatic cells of vertebrates in which the networks frequently attain to a high degree of complexity and in which they often undergo hypertrophy without preliminary fragmentation. Under these circumstances, growth by some process of accretion seems more likely to prevail " (p. 339). As networks themselves have been shown by Cowdry to break up into discrete bodies during cell division it is a little difficult to understand his objection to the breaking up of discrete bodies. If an invertebrate adult cell has say four Golgi bodies, during division each daughter cell will get only two and no amount of accretion can increase the number to four. If Cowdry had said that the growth of the Golgi apparatus differs in vertebrate somatic cells and the invertebrate cells, in the former it being an increase in size and in the latter an increase in number it would have been acceptable for, hypertrophy of the apparatus seen by Gatenby (1917-1919) is by an increase in number and where Bowen (1920) observed a large acroblast, it is formed by the union of a number of small acroblasts and not by accretion.

This lack of clearness about the apparatus tinted by personal bias, the stress laid on technique and the apparatus being spoken of as a substance rather than as a cell organ have made some chromosome workers to squarely dispute its very existence. None of the cytologists have pointed out that Walker and Allen's (1927) criticisms are untenable.

They have evaded the issue by saying that these workers have only proved the composition of the Golgi apparatus. That attitude has led to an ignoring of their work. In their attempt to disprove the existence of the Golgi apparatus Walker and Allen are unconsciously lending themselves to the opinion that such well-established bodies as the chromosomes do not also exist. Walker writes that though "it would seem that much of the work involved in making these very numerous observations upon the Golgi bodies in fixed tissues has been in vain, it may happen that some modifications of an elaborate technique devised to demonstrate something that probably does not exist as such in the living cell, will be of value as a means of advancing our knowledge of chemical changes taking place in cells under different physiological and pathological conditions" (p. 479). In coming to this conclusion Walker and Allen have based their results mainly on experiments with the Golgi apparatus technique on materials supposed to be allied in composition to the Golgi apparatus. But they do not refer to the fact that Cowdry himself has done so with a view to elucidate the composition of the apparatus. "Repeated attempts on my part to make clear canals artificially in mixtures of gelatin and lecithin, fixed by methods designed to reveal the canalicular apparatus, have not been particularly fruitful. Preparations made in this way and stained with iron hæmatoxylin contain canals and vacuoles of many sizes. By careful selection, however, it is possible to gather together a series of canals which resemble to some degree the intracellular formations which are so perplexing" (p. 343). Thus the experiments of Walker and Allen are not novel and hence we shall turn to their other criticisms. According to them what can be seen in the living cells must be considered differently and that one should be quite certain that the cell is in a normal condition. "Probably the nearest approach to normal conditions, in the case of cells of multicellular animals at any rate, is reached in those which are grown *in vitro*. Tissue cells removed from the body and examined immediately cannot be taken as a safe guide, for it is *probable if not certain, that changes involving the production of structures not existing in the normal cell, set in long before death occurs*"[1] (p. 470). According to the above criteria Schneider's discovery of the chromosomes in living material cannot be chromosomes at all because they may be structures produced *de novo*. Tissue culture methods have been attempted only after 1900 and Schneider's observations are on fresh tissue. The whole division of the nucleus into various stages depends upon the acceptance of chromatin as a definite entity. Hence it appears from

[1] Italics are mine.

Walker's sweeping generalisation, that all micro-dissection studies have been in vain. Chambers in his review (1925) comes to the conclusion that the fact " that well differentiated structures are spontaneously beginning to be visible in the nuclei of the third stage, and the fact that these structures stand out with greater clearness on injury, suggest the possibility that the granular chromatin filaments are already present as such in the earlier stages and are not formed *de novo* as an effect of injury. Their invisibility may be accounted for by the identity of their refractive index with that of the nuclear substance in which they lie " (p. 271). Rigid application of Walker's line of argument excludes the acceptance of chromosomes as entities in the nucleus and the other observations on the nucleus are based on fixations by various fixing fluids. Walker is against fixatives because they have been known to separate ingredients from colloidal mixtures. Chemically chromatin is nucleic acid. " It is this which has given the stuff containing nucleic acid the name of the colorable substance, that is, chromatin. The structure which the cytologist calls a chromosome and in which most see the bearer of all herditary traits and some even go so far as to imagine that each trait or character is represented by a distinct unit or gene this structure as shown in fixed dyed section is nothing else than a salt of nucleic acid with the basic dye which has been used to stain it" (p. 75; Mathews, 1925). Thus an analysis of the cell on chemical grounds leaves the histologist or cytologist no criteria on which to base his studies. I have not been able to grasp Walker and Allen's criticism : " The cells are known to contain lipins. If, therefore, the Golgi apparatus is present as such in the living cell, the two structures should both be found in every suitably treated material " (p. 479). This is a rather surprising statement. Any structure present in the cell has to have a composition and the Golgi apparatus being partly lipoidal it is too much to expect a demonstration of the lipoid and the Golgi apparatus in a cell at the same time. It will appear from Walker's criticism that just because cell extracts show the presence of nucleic acid salts any demonstration of chromosomes should necessarily be followed by a demonstration of nucleic acid salts side by side.

Tennent, Gardiner and Smith (1931) have attacked the problem from an entirely different aspect. They extracted from the eggs alcohol soluble substances. In the main, their observations are also similar to those of Walker and Allen. Entire dependence has been placed on technique and the question of morphology and behaviour is not considered at all. Their view is that " there is no specific Golgi substance characteristic of all cells, as chromatin is characteristic of all nuclei " (p. 38). Even in this final statement of theirs it will be seen, criteria applied to the elimination of the

Golgi apparatus as a protoplasmic inclusion are not applied to the chromatin. From the " Chemistry of the Nucleus" (Mathews, 1925) it will be noted that chromatin as such has not yet been isolated. Hence the very term 'chromatin' is a histologists' term for a particular nuclear structure as the Golgi apparatus is the cytologists' term for a cytoplasmic structure. Even the chemical composition of chromatin is not so specific as Tennent, Gardiner and Smith would have us believe. From various cells only two typical acids have so far been isolated. They are yeast nucleic acid isolated from brewer's yeast and wheat; and thymus nucleic acid isolated from the thymus gland, many mammalian tissues and from the fish sperm and other cells. Chromatin apparently consists always of a nucleic acid salt with a protein base. Only one sort of protamin has been observed to occur containing three or four different amino acids.

The chromosomes having such a chemical composition give evidence, according to Chambers, of a definite structure possesing a cortex which can be optically differentiated from a central core during certain stages. " This structure is significant in view of the way in which the artificially induced chromatin filaments come to view in the prophase spermatocyte of the grasshopper. Granules appear out of the hyaline nuclear material, and align themselves in rows. As the granules increase and accumulate, their arrangement about a hyaline non-granular core becomes more and more appreciable. The definitive chromosome finally results by a shortening of the core and the fusion of the granules into a hyaline cortex " (p. 274). This duplex structure is found in Golgi bodies also and Bowen suggested that the Golgi substance is composed of two different materials one lipoid and the other protein in character. Tennent, Gardiner and Smith definitely state that the various 'fractions' studied by them do not represent pure substances and further " that few, if any, of these substances are present in a pure form in the tissues. They are soluble in one another and these mixed solutions may be dispersed in a finely divided condition throughout the cytosome, *often incorporated in a protein base*"[2] (p. 34). Having stated thus they later criticize Bowen's suggestion in the following manner: " Authors who have suggested lipoid and protein as the probable chemical composition of the Golgi apparatus have used a term broad enough to include all the fractions considered in this paper, *i.e.*, the true fats—mixtures of unsaturated and saturated fats—cholesterol and esters, the phospholipins or phosphatids and the galactolipins or cerebrosides. The speculation, therefore, would seem to be safe, but little can be said for its precesion "

[2] Italics are mine.

(p. 38). One wonders how this criticism is consistent with their own statement cited previously. This illogical reasoning is more evident in their criticisms of Cramer and Ludford's (1926) suggestion that the Golgi apparatus is mainly concerned in the synthesis of fat in the intestinal cells during fat absorption. " We know that the particles actively concerned in vital processes of the cell are of a size beyond the limits of observation with the microscope. Products of their activity become visible only when the granules of product come above this horizon " (p. 37). Yet almost immediately they jump to the conclusion that the " diffuse cloud, the formation of fine granules, of chains of granules and finally of larger droplets are the visible expression of one series of vital processes—processes that may be correlated by direct observation with the interaction of the nucleus and cytosome " (p. 37). I cannot at all understand how "vital processes of the cell beyond the limits of observation with the microscope " which they affirm could be correlated by direct observation to the interaction of the nucleus and the cytoplasm could be other than speculation pure and simple. How wholesome and understandable would have been their attitude if they had agreed with Mathews and hinted that chromosomes also do not exist because they are " such museums containing samples of all the chromatin of all the cells of the body, not only all chromatins which develop during life, but all that infinite collection of old masters inherited from the past, and all the infinite number of descendants yet to appear in the eons before us, and presenting qualities usually said to be dormant. They are concealed no doubt in the chromosome attic ready to be produced when occasion arises " (p. 90).

Having shown how unsound are the arguments presented by disbelievers in the existence of the Golgi apparatus I shall finally consider the statement of Strangeways and Canti (1928) that no sign of the Golgi apparatus can be seen in living cells of tissue cultures under dark ground illumination. This statement has been considered by some to be against the presence of the Golgi apparatus in cells. But Strangeways and Canti never saw the nuclear wall and chromatin in the nucleus except in the metaphase. Microdissection studies have shown that the nucleus has a definite wall. In the inter-kinetic nucleus the dark ground illumination reveals no internal structure and so also in the early growing spermatocyte. But Chambers (1925) suggests the possibility that the granular chromatin filaments are already present and are not formed *de novo*. He accounts for their invisibility by the identity of refractive index with that of the nuclear substance in which they lie.

Thus what criticisms are levelled against the existence of the Golgi apparatus are levelled not against that particular structure but against

all modern cytological studies. Their being directed against the Golgi apparatus demonstrates the lopsided logic of the authors of those papers.

What is the Golgi apparatus ? No critical definition has been presented up till now. Hence I shall define the Golgi apparatus in the light of researches carried on up till now bearing in mind that it has a composition, morphology, position in the cell, size, constitution and function :—

1. The Golgi apparatus is a permanent structural element in the cytoplasm universally present in all cells.
2. In quiescent cells generally it has an excentric juxta-nuclear position.
3. It possesses the power of independent growth and multiplication and is passed on from cell generation to cell generation by division processes which have been shown to be of special interest.
4. The Golgi apparatus arises only from pre-existing Golgi and does not arise *de novo*.
5. The apparatus assumes various forms and in invertebrates and germ cells generally it is constituted by discrete bodies having a duplex structure.
6. The apparatus has a lipoidal basis and may also have a second constituent which is protein in character.
7. The Golgi material in many cases is set off rather sharply from the cytoplasmic back-ground and could sometimes be seen in fresh material under ordinary and even dark-ground illumination.
8. The apparatus is consistently impregnated by osmic and silver technique and is resistant to the usual methods of bleaching after osmication.
9. The apparatus shows an increase in size following an increase in volume of the cytoplasm or during active synthesis of visible granules from raw materials in eggs, sperms and secretory cells. This hypertrophy is by an increase in number mainly in invertebrate and germ cells—also in vertebrate lipoidal cells—and by an increase in complexity and size in those cells where networks occur.

Any criticism against the existence of the Golgi apparatus should disprove the validity of the above criteria. No such criticism is yet available and hence I believe that the time for any reiteration of the existence of the Golgi apparatus is long past and attention may well be directed to other problems about which uncertainty still persists.

Acknowledgment.

It is a great pleasure to the author to be able to acknowledge his indebtedness to Professor R. Gopala Aiyar, Director, University Zoological Research Laboratory for his advice and criticism and to the University of Madras for the award of a Research Fellowship.

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CONTRIBUTIONS TO THE EMBRYOLOGY OF THE MENISPERMACEÆ.

I. *Cocculus villosus* DC.

By A. C. Joshi.

(*From the Department of Botany, Benares Hindu University, Benares.*)

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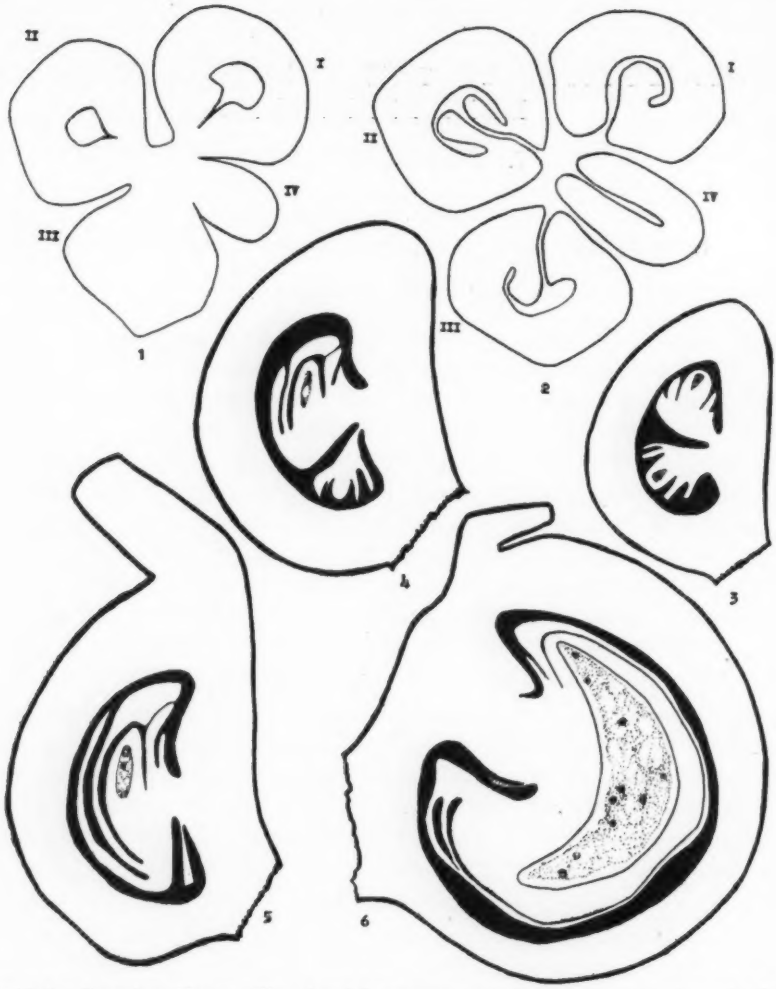
Among the families allied to Magnoliaceæ and Ranunculaceæ, the embryology of the Menispermaceæ is one of the least known. Schnarf (1931) in his book has only mentioned the observations of Ernst (1886) on *Disciphania Ernstii* suspecting the occurrence of apomixis in this species. Since the publication of Schnarf's work, the development of pollen has been described in *Tinospora cordifolia* and *Cocculus villosus* by myself and Rao (1935), but no complete account has appeared so far on the development of the embryo-sac and embryo in any member of the family. A short note on the embryo-sac of *Tinospora cordifolia* was published also by myself and Rao (1934) about two years ago, but a full account of the investigation has still to be given. It is proposed now to complete this work and to report on the embryology of some members of the family common in India.

The present paper deals with the structure and development of the gynœcium, ovule and embryo-sac of *Cocculus villosus* DC. The material required for the study was obtained from a female plant growing near the Benares Hindu University grounds. It was fixed in Bouin's fluid and microtomed according to the customary methods. Sections were stained with Haidenhain's iron-alum hæmatoxylin.

The Gynœcium.

The female flowers of *Cocculus villosus* generally possess three fertile carpels. The occurrence of a fourth carpel, however, is quite common. Rarely this extra carpel is also fertile, but generally, as is shown by Figs. 1 and 2, it is abortive. It does not form any ovary and bears no ovules.

Serial microtome sections of a flower show that the different carpels arise at different levels from the receptacle. This is seen in Figs. 1 and 2, which represent two such sections of a gynœcium at different levels. The carpels according to their origin from below upwards are numbered I, II, III and IV respectively. Their spiral arrangement is thus clearly brought



FIGS. 1-6.—*Cocculus villosus*. Figs. 1 and 2, transverse sections of the gynœcium of a flower at different levels showing the presence of four carpels, one of which (no. IV) is abortive. The carpels are arranged spirally and the numbers I, II, III and IV indicate the order of separation of the four carpels from the floral receptacle. Figs. 3-6, longitudinal sections of carpels at different stages of development. Fig. 3 shows two equally developed ovules at the megaspore mother cell stage. Fig. 4 shows one amphitropous ovule at the 2-nucleate embryo-sac stage and an aborting ovule. Fig. 5 shows a carpel at the time of opening of a flower. There is one amphitropous ovule containing a 7-nucleate embryo-sac and a scale-like remnant of the second ovule. Fig. 6 shows a still older carpel with a campylotropous ovule containing free nuclear endosperm. The second ovule is now quite insignificant, but a minute vestige of it is still present. Figs. 1-5 × 85; Fig. 6 × 45.

out. The abortive carpel bears the number IV and is the last carpel to develop from the receptacle.

These observations lead to the conclusion that the present few-carpellary gynœcium of *Cocculus villosus* has been derived by reduction from a structure consisting of a large number of spirally arranged carpels, such as is found in the Magnoliaceæ and Ranunculaceæ.

The form of the abortive carpel in Fig. 2 corresponds to that of a simple leaf folded upwards. This supports the classical theory of carpel morphology against the new doctrines of Dr. Thomas (1931).

The carpels in the Menispermaceæ are mostly described to be uni-ovular. In *Cocculus villosus* also this is so in the mature condition, but in the early stages two ovules are present in each carpel (Figs. 2 and 3). One ovule arises from each margin of a carpel (Fig. 2) and both continue to develop equally till the differentiation of the megaspore mother cell (Fig. 3), but as the megaspores begin to be formed, growth suddenly stops in the lower ovule. The upper ovule therefore soon becomes much bigger than its companion (Fig. 4), and during its further growth crushes the latter into an insignificant structure (Figs. 5 and 6). The development of the carpels of *Cocculus villosus* in this manner gives a clear proof for the origin of the uni-ovular condition from a bi-ovular stage as a result of suppression of one of the ovules.

The Ovule.

As stated before, one ovule develops from each margin of a carpel. One of these is situated slightly below the other (Fig. 2). As the margins of the carpel fuse, they come to lie nearly in one line and one takes up a position definitely below the other. Both the ovules continue to grow straight till they come to touch the opposite carpel wall. This stops their straight growth and causes them to bend, as has been noted by Reeves (1930) in *Medicago*. One of them turns upwards and the other downwards. The proof for the view that the change in the direction of the growth of the ovules is caused by the pressure exerted by the opposing carpel wall is furnished by the fact that the ovules become flattened at this stage at their apex (Figs. 3 and 9). The bearing of these observations on the origin of the different forms of ovule in the angiosperms will be discussed in a later paper, but it may be pointed out that these support the writer's views on this question briefly reported last year (Joshi, 1935).

The form of the ovule is amphitropous up to the 7-nucleate embryo-sac stage. Later, when the endosperm begins to be formed, it becomes campylotropous (Fig. 6).

There are two integuments. The outer is much smaller than the inner and the latter alone takes part in the formation of the micropyle. Both the integuments are two to three cells thick throughout their development, except that in the mature ovule the inner in the region of the micropyle becomes many-layered. They remain free from each other and the inner from the nucellus up to the time of fertilisation (Fig. 5), and in the chalazal part of the ovule for a short distance even when a good deal of endosperm has been formed (Fig. 6).

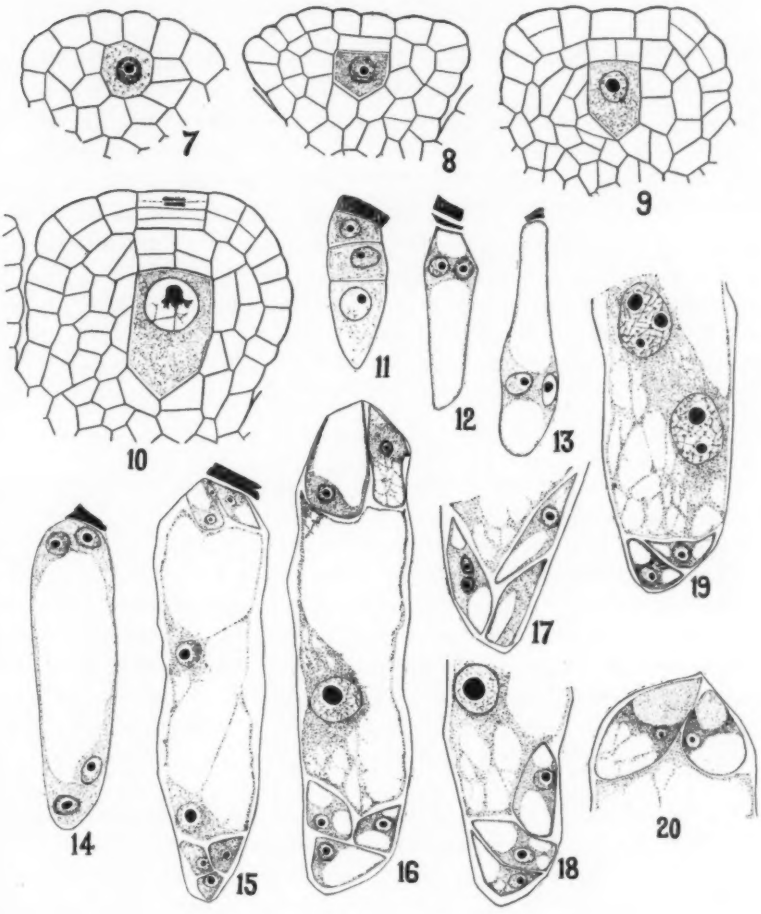
The chief point of interest about the nucellus is the development of a small epidermal cap (Figs. 9 and 10). The development of this begins at the corners of the nucellus about the time the primary wall cell divides anticlinally (Fig. 9). Later this extends to the middle portion of the nucellus (Fig. 10). The thickness of the epidermal cap varies from two to four layers of cells.

Development of the Embryo-sac.

A single archesporial cell differentiates in each ovule (Fig. 7). It cuts off a primary wall cell to form the megaspore mother cell (Fig. 8). The first division in the primary wall cell is anticlinal (Fig. 9). Further divisions take place in all planes and give rise to about six layers of parietal tissue.

The megaspore mother cell undergoes the heterotypic (Fig. 10) and the homotypic divisions in the normal manner and a linear tetrad of megaspores is formed (Fig. 11). The chalazal megaspore gives rise to the embryo-sac and the rest degenerate. Vestiges of the degenerating megaspores are present even when the embryo-sac reaches the 8-nucleate condition (Fig. 15).

The development of the embryo-sac corresponds to the normal type (Figs. 12–15). The bi-nucleate embryo-sac is noteworthy on account of the variation in vocuolation. Sometimes the micropylar vacuole is smaller than the chalazal (Fig. 12). In other cases, it is bigger than the chalazal (Fig. 13). The differentiation of the egg-apparatus and the antipodals takes place nearly simultaneously. The upper polar nucleus is generally the first to move towards the middle of the embryo-sac (Fig. 15), and the two polar nuclei fuse below the middle to form a large secondary nucleus (Fig. 16). The polar nuclei increase appreciably in size after their differentiation, while the size of the other six nuclei of the embryo-sac remains nearly constant (Fig. 15).



FIGS. 7-20.—*Cocculus villosus.* Fig. 7, primary archesporial cell. Fig. 8, differentiation of the primary wall and the megaspore mother cells. Fig. 9, anticlinal division in the primary wall cell and the beginning of epidermal cap formation in the nucellus. Fig. 10 illustrates the structure of the ovule at the time of synizesis in the megaspore mother cell. Fig. 11, linear tetrad of megaspores. Figs. 12 and 13, 2-nucleate embryo-sacs. Fig. 14, 4-nucleate embryo-sac. Fig. 15, 8-nucleate embryo-sac. Fig. 16, 7-nucleate embryo-sac. Fig. 17, antipodals from another mature embryo-sac. One of them is 2-nucleate. Fig. 18, chalazal part of an embryo-sac showing antipodals and a secondary nucleus. Fig. 19, chalazal part of an embryo-sac showing two antipodals and two endosperm nuclei. Fig. 20, micropylar end of an embryo-sac showing two synergids with filiform apparatus. × 700.

The Mature Embryo-sac.

The mature embryo-sac (Fig. 16) is a nearly cylindrical structure about 100μ long. It is 7-nucleate, the polar nuclei in every case fusing at a very early stage. The egg has the usual structure. The synergids show small nooks. Their apices are pointed and a filiform apparatus becomes clearly visible in the later stages (Fig. 20). Their vacuolation and the position of the nucleus is normal. The arrangement and structure of the antipodals is shown by Figs. 15–18. They develop large vacuoles from an early stage. In one case an antipodal cell was found to possess two nuclei (Fig. 17). They often persist up to the early stages of endosperm development. Fig. 19 shows their presence in an embryo-sac containing two endosperm nuclei. No deposition of starch was observed in the mature embryo-sacs.

Summary.

The structure and development of the gynœcium, ovule and embryo-sac of *Cocculus villosus* DC. has been studied.

There are generally three fertile carpels in a flower, but a fourth one is frequently present. The extra carpel is generally abortive. The carpels are arranged in a spiral manner on the floral axis. In the beginning two ovules develop in each carpel. Later the lower one of these is suppressed. These observations show that the few-carpellary gynœcium of *Cocculus villosus* has been derived by reduction from a multicarpellary structure and the uni-ovular carpel from a bi-ovular type.

The functional ovule is amphitropous up to the development of the embryo-sac, but becomes campylotropous during the formation of the endosperm. There are two integuments, which remain free from each other and the nucellus up to the mature embryo-sac stage. The micropyle is formed only by the inner integument. The nucellus is characterised by the development of a small epidermal cap.

The primary archesporium is restricted to a single cell. A primary wall cell is cut off. The megaspore mother cell gives rise to a linear tetrad of megaspores. The chalazal megaspore alone is functional. The development of the embryo-sac corresponds to the normal type. The polar nuclei fuse at an early stage. The synergids possess small hooks and show a clear filiform apparatus in the later stages. The antipodal cells develop large vacuoles.

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ON TWO NEW TREMATODES OF THE GENUS *OPEGASTER* OZAKI, WITH A SYSTEMATIC DISCUSSION ON THE FAMILIES *OPECOELIDAE* OZAKI, 1925 AND *COITOCAECIDAE* OZAKI, 1928.

By K. R. Harshey, M.Sc.

(*From the Department of Biology, M. T. B. College, Surat.*)

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Introduction.

Till 1931 only three species of the genus *Opegaster* Ozaki, namely *Opegaster ovatus*, *O. rectus* and *O. brevifistula*, all described by Ozaki in 1928, were recorded. In 1933 I added another species *O. anguillii* and have suggested that the species *O. ovatus* and *O. rectus* should be merged in one species under the name of *O. ovatus* which has been recognised by its author as the type species of the genus. In this paper are described two new species of the genus *Opegaster* Ozaki, *i.e.*, *Opegaster mastacembalii* n. sp. and *Opegaster mehrii* n. sp. Two to four parasites of the first species were obtained from each of the six fresh-water eels *Mastacembalus armatus* available in the market at Jubbulpore, C.P., in October 1932. Eight specimens of the second species were obtained in February 1933 from the same place and from only one specimen of the above host.

While including *Coitocæcum* Nicoll in the *Opecælidæ* in 1935 Ozaki suggested " The anal canal and anus of *Opecælus* is probably derived from the posterior intestinal arc of *Coitocæcum* by its backward prolongation and the formation of a pore ". The study of all the species hitherto known and the two species of *Opegaster* described in this paper confirm the authenticity of this statement. But the backward prolongation and formation of a pore at the junction of the cæca seems to be derived through the species of *Opegaster* rather than through the species of the genus *Opecælus* for in *Opegaster mehrii* n. sp. the anal canal is totally absent and the anus is situated ventrally in the middle of the intestinal arc. The anal canal is short in *Opegaster mastacenbalii* n. sp. whereas it is long in other species of *Opegaster* and *Opecælus*.

I am grateful to Dr. H. R. Mehra of the Allahabad University, for his valuable guidance in the study of the parasites and help in the preparation of the manuscript.

Opegaster mastacembalii *n.sp.*

The worms are found, in the small intestine, attached to the intestinal wall of the fresh-water eel *Mastacembalus armatus*. The part of the body cephalad to the acetabulum is highly extensible and in fully extended condition it measures almost equal to the post acetabular region.

The worms in living condition are quite transparent, with a deep yellow patch in between the acetabulum and cephalad margin of the ovary due to the colour of the eggs contained in the uterus. The body is ovate and its wall is thin, smooth and entirely devoid of spines. Measurements in millimetres of the body, suckers, digestive and reproductive organs are given in Table I.

TABLE I.

1. Length of body	1·39—1·56	9. Oesophagus	Short
2. Breadth of body	0·35—0·48	10. Distance between two suckers	0·19
3. Ratio of length to breadth of body .. .	3·585 : 1	11. Length of ovary	0·85
4. Diameter of oral sucker ..	0·155	12. Breadth of ovary	0·22—0·23
5. Diameter of ventral sucker ..	0—275	13. Length of anterior testis ..	0·19—0·22
6. Number of papillae on ventral sucker	Six	14. Breadth of anterior testis ..	0·25—0·31
7. Ratio of oral to ventral sucker	1 : 1·774	15. Length of posterior testis ..	0·221
8. Diameter of pharynx	0·085	16. Breadth of posterior testis ..	0·31

The oral sucker is terminal and smaller than the ventral sucker; the ratio of oral to ventral sucker is 1 : 1·774. The latter is situated in the second quarter of the body 0·19 mm. distance from the oral sucker and a little behind the intestinal fork. It has thick protruding muscular walls provided with six finger-like papillæ. A very short prepharynx, bulbus pharynx and a short œsophagus, with almost uniform breadth are present. The intestinal cæca converge centrad, behind the posterior testis and unite with each other in the median line close behind the caudal margin of the intracæcal vitellaria in the last fifth of the body, to form a short backwardly directed common cæcum of 0·017 mm. length, which opens to the exterior at the anus, situated ventrad, a little anterior to the subterminal excretory pore.

The reproductive organs form a compact mass, situated in the posterior half of the body. The triangular or globular testes of almost equal size are situated in tandem or slightly obliquely close to each other. They are transversely broad and have entire or somewhat irregular outline. The vasa efferentia arise from the middle of the anterior margin of the testes and run cephalad to form a very short vas deferens in the middle line close behind ventral sucker. The vesicula seminalis externa, with its thin membranous walls, is a long tube of almost uniform breadth and has one bend near the middle or terminal end between the acetabulum and the intestinal bifurcation. It is situated to the right side of the acetabulum or overlaps dorsally. Its anterior part runs cephalad, obliquely to the left side of the body to enter a very small but conspicuous cirrus sac, situated to the left side a little in front of the intestinal bifurcation.

The ovary is much broader than long and has entire margins ; it occupies a median or submedian position 0·221 to 0·36 mm. distance behind the caudal margin of the ventral sucker. The oviduct arises from about the middle of the anterior margin of the ovary and runs anteriorly parallel to the body wall for a little distance before it runs centrad to enter the shell gland mass, which is situated median close in front of the ovary. A short Laurer's canal is present. The genital pore lies to the left side near the level of the pharynx. A conspicuous receptaculum seminis uterinum, filled with sperms, is present. The uterus has a few transverse coils in between the ovary and the caudal margin of the acetabulum. The number of eggs varies from 12 to 20.

The vitellaria are highly developed extending from the posterior end of the body to the level of the middle of the pharynx. They are united immediately behind the posterior margin of the posterior testis to form the characteristic intercæcal vitelline mass which lies between the latter and the intestinal arc 0·10 mm. distance in front of the hinder end. The vitallaria are also united, close behind acetabulum, to form a transverse connecting band, the presence of which distinguishes this species from all the other species of the genus. The vitelline follicles are oval or rounded in shape. The position and arrangement of the vitelline ducts are similar to those of *Opegaster anguillii* described by me.

The excretory system as studied in the living condition of the specimens shows a close resemblance to that of *Opegaster anguillii* Harshey, and other species of the genus. The excretory pore, however, is situated a little distance in front of the hinder end and not at the terminal end as in other species of the genus.

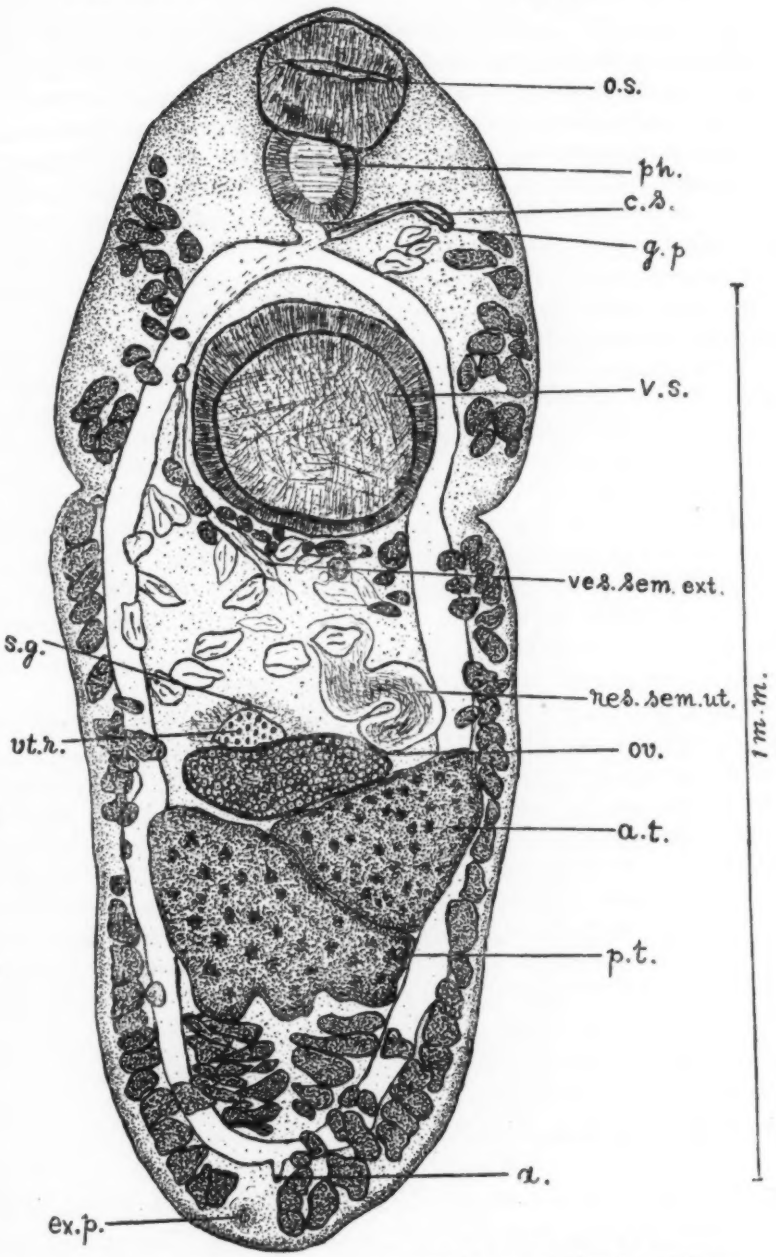

FIG. 1. Ventral view of *Opegaster mastacembalii* n.sp. showing the anatomy.

Discussion.

The new species *Opegaster mastacembalii* differs remarkably from *O. ovatus* Ozaki, in the presence of finger-like papillæ on the ventral sucker, in having unlobed testes and lastly in the presence of a transverse connecting band of vitellaria close behind the acetabulum. In the last feature it is distinguished from all other hitherto known species of the genus. It also differs from *Opegaster parapristipomati* Yamaguti (1934) in the position, shape and size of its gonads, in the presence of a short œsophagus and in the presence of well-developed papillæ on the acetabulum. *Opegaster mastacembalii* n.sp. resembles *O. syngnathi* Yamaguti (1934) in the number of papillæ on the acetabulum but it differs from it in the position, shape and size of its gonads and in the extent of vitellaria, which are rudimentary near the level of intestinal bifurcation in the latter species. It also resembles *O. brevifistula* Ozaki, in the number of papillæ on the ventral sucker but, however, differs from it in the position, shape and size of its gonads and in the presence of an œsophagus. It closely resembles *Opegaster anguillii* Harshey, in the small number of uterine coils with relatively smaller number of eggs, which are devoid of a stumplike projection, but it differs from it in the number of papillæ (six in *O. mastacembalii* n.sp. and four in *O. anguillii*) on the ventral sucker. The ratio of oral to ventral sucker also differs in all the species.

Diagnosis of Opegaster mastacembalii *n.sp.*

Body length 1·39 to 1·59 mm.; maximum width 0·35 to 0·47 mm. in the acetabular region. Oral sucker terminal. Short prepharynx and a bulbus pharynx are present; a short œsophagus present. Ventral sucker provided with six finger-like papillæ. Gonads form a compact mass in posterior half of body; ovary broader than long, median or submedian; entire testes of almost equal size, tandem or oblique. Vitellaria extending from the hinder end of body to middle of pharynx with a transverse connecting band close behind acetabulum. Uterus with few transverse coils between caudal margin of acetabulum and ovary; ova without a stumplike projection 12 to 20 in number; size of eggs 0·0595 by 0·030 mm. Excretory pore subterminal.

Host.—*Mastacembalus armatus.*

Location.—Small intestine.

Locality.—Jubbulpore, C.P. (India).

Opegaster mehrii *n.sp.*

Body ovate, 2·46 to 3·43 mm. in length and 0·88 to 1·16 mm. in its breadth in the region of ventral sucker; cutical smooth. The terminal oral

sucker 0·17 to 0·23 mm. in diameter. Acetabulum, 0·33 to 0·41 mm. in diameter, sessile with six conspicuous marginal papillæ, situated in the anterior half of second quarter of the body length. Short prepharynx present ; pharynx 0·15 to 0·17 mm. in length and 0·14 to 0·15 mm. in breadth ; œsophagus 0·19 mm. long and 0·034 mm. wide ; intestinal cæca uniting with each other a little anterior to the posterior extremity ; anal canal absent ; anus median situated on the intestinal arc.

Testes and ovary contiguous in third quarter of body length ; testes globular to ovoid, with a somewhat irregular margin ; anterior testis 0·229 by 0·45 mm. slightly displaced to the left. Small cirrus sac present ; vasicula seminalis externa extending posteriorly to level of receptaculum seminis uterinum.

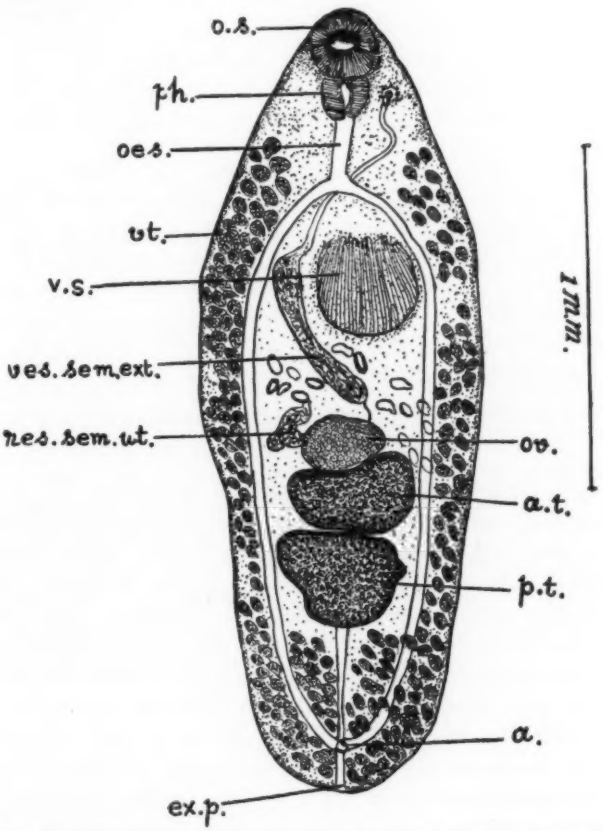

Fig. 2. Ventral view of *Opegaster mehrii* n.sp. showing the anatomy.

Ovary median, irregularly ovoid, 0·16 to 0·26 mm.; shell gland mass anterior to ovary slightly to the left side; receptaculum seminis absent; Laurer's canal present. Vitellaria lateral, extending from middle of œsophagus to posterior end, filling entirely the post testicular part of the body except the excretory bladder. Genital aperture to the left side near oral sucker. Uterus intercæcal between ovary and genital pore; eggs, 0·075 by 0·034 mm., without a stump-like projection.

Excretory bladder in the form of simple tubular sac extending forward to the shell gland mass and opening terminally at hinder end.

Discussion.

The new species *Opegaster mehrii* differs from all the known species of the genus in the absence of an anal canal which may be regarded as an important and characteristic feature of this species. *O. mehrii* n.sp. differs from *O. ovatus* in the unlobed character of its testes and in the presence of papillæ on the acetabulum. In the number of papillæ on the acetabulum it resembles *O. brevifistula*, *O. mastacembalii* n.sp., and *O. syngnathi* Yamaguti, but the œsophagus is absent in *O. brevifistula*, short in *O. mastacembalii* n.sp., and long in *O. syngnathi*. Although the new species *Opegaster mehrii* resembles *O. syngnathi* in having a long œsophagus but it differs from it in the extent of vitellaria. It also differs from *O. anguillii* Harshey in the number of papillæ on the ventral sucker.

Host.—*Mastacembalus armatus.*

Location.—Intestine.

Locality.—Jubbulpore, C.P. (India).

Systematic Discussion on the Families Opecœlidæ *Ozaki*, 1925 *and* Coitocæcidæ *Ozaki*, 1928.

Ozaki in 1928 in agreement with Odhner recognised the close affinities between *Opecœlus* and *Podocotyle* but retained the family *Opecœlidæ* which, according to him, differs from *Allocreadiidæ* in the united cæca, with an anus, in the presence of a small cirrus sac, absence of receptaculum seminis and presence of a vesicula seminalis externa. Tubangui (1928) also retained the family *Opecœlidæ* while describing his new species *Opecœlus minimus*. He, in 1931, created the genus *Orientocreadium* for *Orientocreadium batracoides* including it in the *Allocreadiidæ* although vesicula seminalis externa is present and receptaculum seminis absent in the genus. Recently, Manter (1934) erected the genus *Opecœlina* for *Opecœlina scorpænæ* and *Opecœlina helicoleni*, distinguishing it from all the genera of the family on account of the presence of a large cirrus sac and a receptaculum seminis. He reduced

the family *Opecœlidæ* to the rank of a sub-family in the *Allocreadiidæ* with which I entirely agree because the union of the intestinal cæca at the hinder end and the presence of an anus are the only features distinguishing the *Allocreadiidæ* from the *Opecœlidæ*. Just as no separate families are needed and created for *Balforia* and *Chaunocephalus* which though they possess an anus or ani, resemble in all important features the *Echinostomidæ*, no family need be created for *Opecœlus* and its other closely allied genera hitherto included in the *Opecœlidæ*.

In 1934 Yamaguti created two new genera *Podocotyloides* and *Pedunculacetabulum* for his new species *Podocotyloides pentalophallus* and *Pedunculacetabulum opisthorchis* respectively and included them in the family *Allocreadiidæ*. Although these two genera widely differ from each other possess an acetabulum situated on a pedicle which is broad based in *Podotyloides* and long in *Pedunculacetabulum*. It appears to me now that the genus *Opecœlus* Ozaki comes closer to *Podocotyloides* than to the genus *Podocotyle*, which stands in close relation to the former, for the genera *Opecœlus* and *Podocotyloides* possess a pedunculate acetabulum whereas it is sessile in the genus *Podocotyle*.

Though Nicoll, in 1915, created the genus *Coitocœcum* for *Coitocœcum gymnophallus* he was uncertain about its systematic position. He hinted upon its superficial relationships with the *Allocreadiidæ* but at the same time considered the absence of a cirrus sac and condition of the intestinal cæca in this genus as sufficiently important to exclude it from that family. Poche, in 1926, assigned definitely to the *Allocreadiidæ*, creating a new subfamily *Coitocœcinæ* for it. In 1925, Ozaki established the family *Opecœlidæ* to which he assigned the genera *Opecœlus* Ozaki, and *Coitocœcum* Nicoll; three years later he added to this family two more genera *Opegaster* and *Anisoporus* and created the family *Coitocœcidæ* for *Coitocœcum* Nicoll, on account of the absence of an anus which he considered as a notable and important character. Ozaki, in a subsequent paper in 1928, described five species of *Coitocœcum*, namely, *C. latum*, *C. orthorchis*, *C. diplobulbosum*, *C. plagiorchis* and *C. unibulbosum* and gave a definition of the family *Coitocœcidæ*. Winfield (1929) following Ozaki removed *Coitocœcinæ* Poche (1926) from the family *Allocreadiidæ*.

TABLE II.

Allocreadiidæ	*Coitocæcidæ*	*Opecælidæ*
1. Fish trematodes.	Fish trematodes.	Fish trematodes.
2. Spines present or absent.	Spines absent.	Spines absent.
3. Acetabulum smaller or larger than oral sucker, pedunculate or sessile, with or without papillæ.	Acetabulum larger than oral sucker, without papillæ and sessile.	Acetabulum larger than oral sucker, with or without papillæ, pedunculate or sessile.
4. Position of genital pore variable, from behind intestinal fork to level of pharynx, median or lateral.	Position of genital pore definite, lateral near pharynx.	Position of genital pore definite, lateral near pharynx.
5. Highly developed cirrus sac present or absent.	Small cirrus sac present.	Small or large cirrus sac present.
6. Vesicula seminalis externa present or absent.	Vesicula seminalis externa present.	Vesicula seminalis externa present.
7. Receptaculum seminis present or absent.	Receptaculum seminis absent.	Receptaculum seminis present or absent.
8. Intestinal cæca separate.	Intestinal cæca united posteriorly and without anus.	Intestinal cæca united posteriorly and with anus.
9. Ovary anterior to testes.	Ovary anterior to testes.	Ovary anterior to testes.
10. Uterus anterior to ovary or extending behind it to hinder end of body.	Uterus anterior to ovary.	Uterus anterior to ovary.
11. Vitellaria highly developed, contiguous or non-contiguous in post testicular zone.	Vitellaria highly developed contiguous in post testicular zone.	Vitellaria highly developed, contiguous in post testicular zone.
12. Excretory bladder simple tubular sac.	Excretory bladder simple tubular sac.	Excretory bladder simple tubular sac.

In view of what has been said and from the above table the family *Coitocæcidæ* also cannot be maintained because *Coitocæcum* differs from *Opecælidæ* only in the absence of an anus. We accordingly drop the families *Opecælidæ* and *Coitocæcidæ* and maintain for all the genera contained therein the sub-family *Opecælinæ*, which we, following Manter, include it in the family *Allocreadiidæ*. An emended definition of the sub-family *Opecælinæ* is given below.

Opecælinæ.

(Sub-family diagnosis).

Allocreadiidæ.—Worms of small to medium size, with round or oval or cylindrical body and smooth cuticula. Oral aperture terminal or sub-terminal. Acetabulum pre-equatorial, larger than oral sucker, pedunculate or

sessile, with or without papillæ. Lateral or suctorial cup and genital sucker present or absent. Prepharynx and pharynx present; œsophagus present or absent; cæca uniting to form an arc in the hind body with or without anus. Genital pore preacetabular, lateral. Testes post acetabular, post uterine, post ovarian and intercæcal. Small or large cirrus pouch present, not completely enclosing the vesicula seminalis. Ovary post acetabular, post uterine, pretesticular, intercæcal, median or sub-median. Laurer's canal present. Receptaculum seminis present or absent. Initial part of uterus forming a receptaculum seminis uterinum; uterine coils transverse, pretesticular and intercæcal. Vitellaria with small or well-developed follicles, lateral uniting behind testes. Excretory vesicle simple, elongated, tubular sac extending forward to the shell gland complex and with a terminal or sub-terminal pore. Parasites of fishes.

Key to the Genera of the Sub-family Opecœlinæ.

1. Anus absent	*Coitocæcum* Nicoll.
2. Anus present	3.
3. Genital sucker and lateral cup present ..	*Anisoporus* Ozaki.
4. Genital sucker and lateral cup absent ..	5.
5. Body ovate; vitellaria near intestinal fork or extending into the neck	*Opegaster* Ozaki.
6. Body elongated; vitellaria entirely post acetabular	7.
7. Receptaculum seminis present	*Opecœlina* Manter.
8. Receptaculum seminis absent	*Opecœlus* Ozaki.

Key to Species of the Genus Opegaster *Ozaki.*

Body ovate or elongated; vitellaria extending upto the intestinal fork or into the neck; acetabulum with or without finger-like papillæ; anus with or without anal canal..	*Opegaster* Ozaki.
1. Acetabulum without papillæ	*O. ovatus* Ozaki.
2. Acetabulum with inconspicuous papillæ ..	*O. parapristipomatis* Yamaguti.
3. Acetabulum with conspicuous papillæ ..	4.
4. Papillæ four in number	*O. anguillii* Harshey.
5. Papillæ six in number	6.
6. Oesophagus absent	*O. brevifistula* Ozaki.
7. Oesophagus present	8.
8. Anal canal absent	*O. mehrii* n.sp.

9. Anal canal present .. .. .. 10.
10. Vitellaria with transverse connecting band close behind acetabulum .. .. *O. mastacembalii* n.sp.
11. Vitellaria without transverse connecting band close behind acetabulum .. *O. syngnathi* Yamaguti.

Summary.

It is suggested that the genes *Opecœlus* Ozaki comes closer to *Podocotyloides* Yamaguti, than to the genus *Podocotyle.*

Two new species of Opegaster, *O. mastacembalii* and *O. mehrii* from the fresh-water eel *Mastacembalus armatus* are described; and the specific diagnosis of *O. mastacembalii* is given. For convenience of treatment a detailed description of *O. mehrii* is avoided. A key to the species of the genus *Opegaster* is given.

In the concluding part of the paper it is pointed out that the families *Opecœlidæ* and *Coitocæcidæ* should be dropped and all the genera contained therein be included in the sub-family *Opecœlinæ* which Manter includes in the family *Allocreadiidæ.* The definition of the sub-family *Opecœlinæ* is emended and a key to genera of the sub-family *Opecœlinæ* is given.

EXPLANATION OF LETTERING.

a.	Anus.	*ph.*	Pharynx.
a.t.	Anterior testis.	*res. sem. ut.* ..	Receptaculum seminis uterinum.
c.s.	Cirrus sac.		
ex. p.	Excretory pore.	*s.g.*	Shell gland.
g.p.	Genital pore.	*p.t.*	Posterior testis.
i.c.	Intestinal cæca.	*v.s.*	Ventral sucker.
oes.	Oesophagus.	*ves. sem. ext.* ..	Vasicula seminalis externa.
o.s.	Oral sucker.	*vt.*	Vitellaria.
ov.	Ovary.	*vt.r.*	Vitelline reservoir.

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THE MORPHOLOGY AND SYSTEMATIC RELATIONSHIPS OF A NEW BOLOCEROIDARIAN FROM BRACKISH-WATER NEAR MADRAS, TOGETHER WITH AN ACCOUNT OF ITS ASEXUAL REPRODUCTION.

By N. Kesava Panikkar, M.A., M.Sc.

(From the University Zoological Research Laboratory, Madras.)

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Introduction.

The present communication deals with an interesting anemone, obtained from the Adyar backwaters in addition to the other brackish-water Actinians which formed the subject of two previous studies (Panikkar, 1936 and 1937). This belongs to the sub-tribe Boloceroidaria, but, as will be shown later, is different from the genera of Boloceroidaria already described. The anemone is hence referred to a new genus for which the name *Boloceractis* is suggested. The anatomy of the type species, *Boloceractis gopalai*, and the peculiar method of asexual reproduction by tentacular regeneration are described in this paper, a preliminary account of which was read before the 1936 (Indore) session of the Indian Science Congress.

Generic and Specific Diagnoses.

Sub-tribe	..	Boloceroidaria,	Carlgren, 1924.
Family	..	Boloceroididæ,	Carlgren, 1924.

Boloceractis, Gen. Nov.

Boloceroididæ with short column and distinct basal disc without basilar muscles. Column broader in the distal part than the proximal, smooth, without spirocysts but with a few nematocysts, and without stinging warts, vesicles or other structures characteristic of *Bunodeopsis*. Tentacles numerous; those of the innermost circle the largest. They are deciduous and provided with sphincters at their bases. Mesenteries more numerous in the distal part than in the proximal. The primary mesenteries with very weak and diffuse retractors, about six pairs or a few more in number and sterile. Distinct siphonoglyphes present. Imperfect mesenteries in large

numbers, and the gonads usually borne by them. No sharp distinction of the mesenteries into macrocnemes and microcnemes. Columnar sphincter absent. Type species *B. gopalai*.

Boloceractis gopalai, n.g. et sp.*

Diagnosis.—Pedal disc distinct, yet variable in thickness. Tentacles $6 + 6 + 12 + 48$....variable in number according to the age of the individual. The number of tentacles in each cycle may vary according to the number of mesenteries. Innermost endocœlic tentacles the largest and mostly deciduous. Oral disc narrow with prominent mouth. Actinopharynx well developed and having distinct siphonoglyphes with aboral prolongations. Pairs of mesenteries (6–8), (6–8), (12–16), (24–32).....; the mesenteries of the first order perfect and without gonads usually. Retractors very feeble. Imperfect mesenteries always fertile and with filaments; these mesenteries are more numerous near the oral disc than below. Sexes separate. Nematocysts of the tentacles curved and of the ordinary type. The sizes of the nematocysts are as follows :—

Nematocysts of the column	..	..	9–13 $\mu \times$ 2–4 μ.
Nematocysts of the tentacles	(1)	..	46–49 $\mu \times$ 6–7 μ.
,, ,, ,,	(2)	..	19–21 $\mu \times$ 4–5 μ.
Nematocysts of the filament	..	..	33–35 $\mu \times$ 6–7 μ.
Spirocysts of the tentacles	..	..	27–36 $\mu \times$ 5–6 μ.

Asexual reproduction is very common in this speices.

Morphology of the Anemone.

The anemones are small, with short columns, from 5 to 10 mm. in height, slightly broader in the oral disc region (5 to 8 mm.) than the basal part (5 to 6 mm.). The tentacles are very prominent and surround the column making the latter look insignificant.

The Base.—The basal disc of the anemone, though distinct and very well developed in certain specimens, is always without basilar muscles. There is considerable variation in the general shape of the basal disc; in most specimens it is flat and adhesive, while in others it is cup-shaped.

The Column.—The column wall is mostly thin and some specimens reveal the insertions of the mesenteries from the outside. The column is smooth and without spirocysts so far as could be ascertained from the material available; in this respect, this species is in sharp contrast to the

* I have much pleasure in associating the species with the name of Prof. R. Gopala Aiyar, Head of the Zoology Department of the University of Madras.

members of the genus *Boloceroides*. The nematocysts are very scarce on the column ; they are few and scattered and are of a small size (compare diagnosis). Cinclides are absent.

There is no sphincter, either endodermal or mesogleal. The longitudinal ectodermal muscles of the column attain considerable development. In sections, they form a prominent layer lying next to the mesoglea. This musculature is uniformly developed throughout the column.

The ectoderm of the column is a comparatively thin layer, with rather sparse mucuos cells. The endoderm is also a thin and compact layer, often containing zooxanthellæ in large numbers. The mesoglea does not appear to contain scattered nuclei.

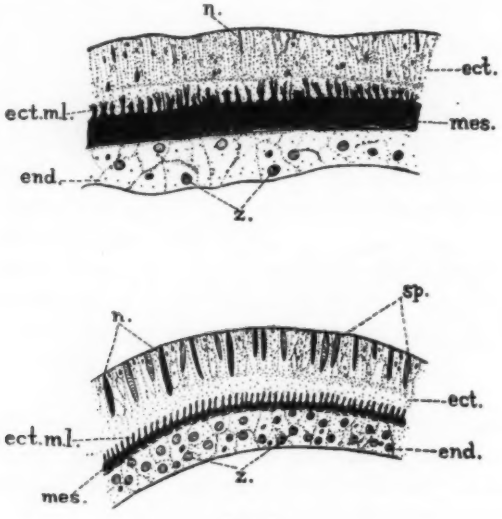


FIG. 1.

The Oral Disc.—The disc is thin and depressed, with the mouth placed on a central, raised, prominence. The throat is not open and the ridges are not distinct. Since the tentacles occur in crowded cycles surrounding the mouth, the margin of the disc is not clear. The oral disc is usually 6 to 8 mm. broad. The ectoderm, mesoglea and the endoderm of the oral disc are very thin and are scarcely recognizable as separate layers.

The Tentacles.—Large numbers of tentacles are present and they are smooth, long and pointed. They crowd at the distal extremity of the column and are usually 8 to 14 mm. in length. In younger specimens, they may be recognized as belonging to five or six cycles. The innermost

or the first cycle of six to eight tentacles corresponds to the primary endocœls and consists of the largest. The length of the tentacles gradually diminishes from the inner to the outer cycles. As the outer cycles contain large numbers of tentacles, especially in older individuals, it is not possible to distinguish the several cycles. Judged from many specimens, the theoretical arrangement seems to be $6 + 6 + 12 + 24 + 48 + 96.....$ The number varies according to the age and size of the anemone. In very young examples, all the cycles are not observed, while the asexually reproduced individuals exhibit irregularities of tentacular arrangement.

In structure, the tentacle is characterised by having a very weakly developed mesogleal layer. The longitudinal musculature of the tentacles is ectodermal. The ectodermal and endodermal layers are thin, and the cells of the latter often teem with zooxanthellæ.

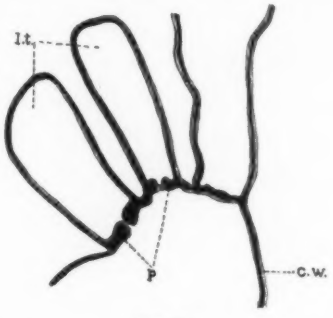


FIG. 2.

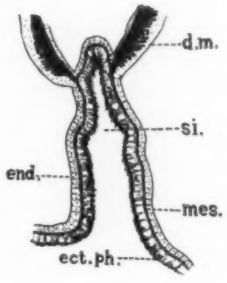


FIG. 3.

The bases of the tentacles are provided with sphincters projecting into the space where the tentacular cavities communicate with the cœlenteron. The mesoglea of the sphincter is thickened towards the inner edge of the partition between the cœlenteron and the lumen of the tentacle, and bears the muscle processes which control the action of the sphincter. As compared with similar sphincters described for *Boloceroides hermaphroditica*, *Bunodeopsis globulifera* and *Bolocera tuediæ* (Horton, 1934; and Stephenson, 1935), it is clear that the basal sphincter mechanism of this anemone is fairly well developed and capable of completely closing the communication between the tentacular cavity and the cœlenteron. The deciduous nature of the tentacles is closely associated with the development of this sphincter. As in all tentacular sphincters, the muscle fibres impinge on the endoderm and the sphincter should hence be reckoned as endodermal.

Since the actual body of the anemone is much smaller than the oral disc and the tentacles, the latter structures are not retractile.

The Actinopharynx.—The stomodæum extends to the middle of the cœlenteron. Sections of the actinopharynx show distinct siphonoglyphes accompanied by directives; but the number of directives and the siphonoglyphes is variable because of the prevalence of asexual reproduction. In structure, the siphonoglyphes do not present an advanced condition since their lining cells are not highly specialized. Mucous cells are found in the ectoderm of the actinopharynx.

The Mesenteries.—The number of mesenteries is highly variable according to the age and size of the anemone. They are often numerous and a sharp distinction into microcnemes and macrocnemes does not exist. The primary mesenteries, which are the prominent ones, number six to eight pairs, and they differ from others in having well-developed mesenterial filaments and long, feeble retractors. The directive mesenteries also belong to this cycle, and as mentioned above, their number varies; at least a pair is always present.

The mesenterial musculature is very weakly developed. The parietal muscles are completely absent on all the mesenteries. The retractors which

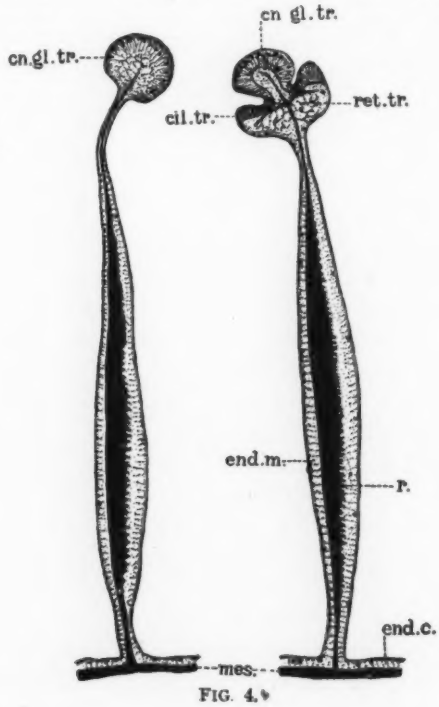


FIG. 4.

are present only on the primaries have feeble muscle processes. The mesenterial muscles are best developed near the basal part of the anemone.

As can be expected from the very large number of tentacles, the number of imperfect mesenteries increases near the oral disc of the column from what it is below. Their arrangement is not always regular. Curiously enough, most of the imperfect mesenteries near the oral disc bear well-developed mesenterial filaments which are identical in structure to that of the primaries. The tracts in the filaments are fairly well differentiated, with distinct cnidoglandular, reticulate and ciliated regions. The trifid part of the filament is very short as the ciliated tract exists only for a short distance, the remaining part of the filament consisting entirely of the cnidoglandular tract.

The gonads are borne by all the imperfect mesenteries, though rarely the perfect ones are also fertile. The usual condition is that the latter are all sterile, while a distinct reproductive region is often found even on the smallest imperfect mesentery of a fully-grown anemone. The sexes are separate. In the females, the large eggs lie in the mesoglea of the imperfect mesentery.

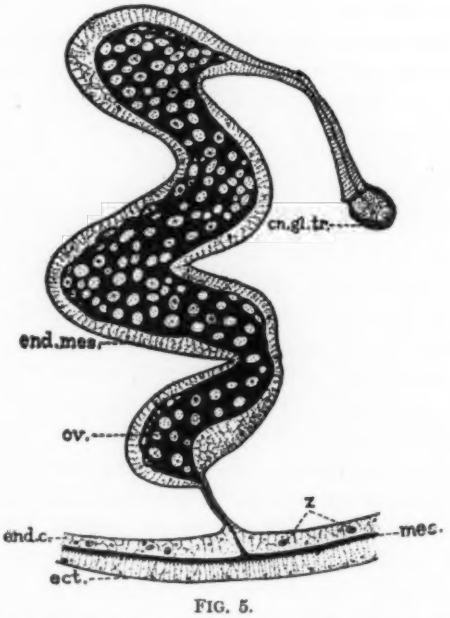


FIG. 5.

Thread Cells.—Cnidæ are very scarce on the base and column. Spirocysts appear to be absent on the column. Though several maceration

preparations were made and carefully examined, no evidence could be obtained to demonstrate clearly the occurrence of spirocysts on the column. These preparations showed only the small columnar nematocysts, which, as pointed out before, are rather scarce. The spirocysts and nematocysts are best developed on the tentacles. The nematocysts of the tentacles are curved in a characteristic manner and the thread of the capsule is of a forked appearance when not exploded, being similar to the capsules figured by Carlgren (1924) for *Boloceroides* and *Bunodeopsis*. The tentacular nematocysts seem to be mostly of the penicillous type. They are indistinctly grouped on the ectoderm of the tentacles, but they do not form definite stinging warts as in *Bunodeopsis*. The spirocysts of the tentacle occur in groups at the base and middle while the nematocysts crowd near the tip.

Asexual Reproduction.

The powers of regeneration and asexual reproduction are developed to a remarkable degree in this anemone. The loss of tentacles, tears on the column, oral disc and the base, are all soon rectified by the regenerative activity of the adjacent tissues. The process of asexual reproduction is also a variant of regeneration as reproduction by longitudinal or transverse fission of the anemone has not been observed. The deciduous tentacles of the anemone regenerate new individuals as in *Boloceroides*. The tentacles remain turgid and swollen even after their separation from the anemone. This is caused by the contraction of the tentacular sphincter which comes away along with the separated tentacle and completely closes up the opening at its base. New tentacles begin to arise from the basal part of the parent tentacle. Their orientation in relation to the column of the growing anemone is the same as in *Boloceroides*. As the growth of new tentacles occasionally begins even before the complete detachment of the parent tentacle, the oral disc of some specimens examined bears a few developing individuals, each in association with a tentacle. This gives a characteristic clustered appearance to the discs of these anemones. The development of the young individuals does not proceed much under this condition. These ' buds ' easily get detached long before they develop into young anemones. The number of such developing young ones carried by such an anemone varies from three to six. Specimens that bear asexually developing young ones do not have ripe germ cells in their mesenteries and the gonads are poorly developed. This observation seems to suggest that the asexual and sexual methods of reproduction do not take place simultaneously and that the two phases alternate.

Asexual reproduction in *Boloceroides* has been the subject of detailed study by Okada (1926, 1930), and Okada and Komori (1932). The asexual

reproductive process in *Boloceractis* does not seem to be so highly evolved as in *Boloceroides*. Okada and Komori discovered in the latter a regular organised process of budding whereby numerous buds are produced around the oral disc, which later grow into new Actinians after liberation. They also observed active tentacular regeneration. In *Boloceractis* we do not find budding taking place in the same way as described for *Boloceroides*. The superior and inferior buds and their peculiar arrangement on the disc of the parent anemone are features not noted in the present form. But it was described above that occasionally, the growth of additional tentacles belonging to the new anemone takes place before the complete detachment of the parent tentacle. This phenomenon is on a small scale comparable to the budding of *Boloceroides*. The young individuals that may develop in this manner in association with the mother anemone get detached at a much earlier stage in *Boloceractis*, as the few new tentacles are all the structures that are developed while on the parent. No mesogleal musculature has been observed in *Boloceractis gopalai* either in association with the developing 'bud' or at the base of the tentacle. The occurrence of a mesogleal musculature as described by Okada and Komori for *Boloceroides sp.* is extermely doubtful (*cf.* Carlgren, 1933).

The order of appearance of the mesenteries in the asexually reproduced individuals could not be studied from the material available.

Bionomics and Distribution.

Boloceractis gopalai has been obtained from the Adyar and the Ennur backwaters near Madras, and from the Astamudi Lake in Travancore. From its known distribution, it would appear that the species is a euryhaline sea anemone.

Specimens have been taken from algal masses as also from the surface of the water since the anemone occasionally floats. It is capable of creeping and swimming movements; it does not, however, appear to be so actively swimming an anemone like *Boloceroides hermaphroditica*. The tentacles are adhesive and helps the anemone to cling to algal masses. Specimens are of an yellowish brown colour in the fresh state.

The exact role played by the tentacular sphincter of the Boloceroidaria and of the other Actiniid genera like *Bolocera* and *Liponema* is by no means clear (*cf.* Stephenson, 1935). It has been suggested by Horton (1935) that the sphincter, by strengthening the tentacular bases, may be of some advantage to swimming anemones like *Boloceroides* (Carlgren, 1900; Komori, 1932), though he could not conceive of a definite mechanism whereby the basal sphincter helps the lashing movements of the tentacles. He was led

to this view by the greater prominence in the sphincter of the free-swimming Actinian *Boloceroides hermaphroditica*, as compared to the similar structures observed in *Bolocera longicornis* and *Bunodeopsis globulifera*. *Boloceroides*, *Boloceractis* and *Bunodeopsis* are non-attached anemones; they have comparatively short columns and the mouth is often wide and open. The partitions at the bases of the tentacles appear to be advantageous in another way. By the contraction of the tentacular sphincter, the communication between the cœlenteron and the tentacular cavity is cut off, and this makes the tentacles turgid, giving it sufficient strength to execute lashing movements effectively. I have observed similar lashing movements even in a species of *Bunodeopsis* from the Madras Harbour, and it is worthy of note that this is not at all a free swimming anemone. Owing to the peculiar habit of shedding the tentacles, several circular openings are often seen on the discs of *Boloceractis gopalai* and the above-mentioned species of *Bunodeopsis*, allowing free communication between the cœlenteron and the outside. Under such circumstances, it naturally suggests that the turgid state of the tentacles necessary to bring about active movements cannot be maintained but for the partitions at their bases. It should be mentioned, however, that as pointed out by Stephenson (1935) for *Bolocera tuediæ*, the real significance of the "tentacle-shedding habit" of these anemones is not at all clear, though in *Boloceroides* and *Boloceractis* this habit has an important bearing on asexual propagation.

Discussion.

The genera *Boloceroides* and *Bunodeopsis* have occupied different positions in the schemes of classification adopted by different actinologists. Taking their many essential Protanthean characters into consideration, Carlgren (1900) referred *Boloceroides* to the Protantheæ along with *Gonactinia*. The differences between *Boloceroides* and *Protanthea* were, however, emphasised by Carlgren. The same arrangement was followed by Pax (1914) and Poche (1914). This position was questioned by Stephenson (1921, 1922) who created the new family Myonanthidæ, to include the four genera *Boloceroides*, *Myonanthus*, *Macrodactyla* and *Nevadne*. *Bunodeopsis*, on the other hand, has been classed along with *Alicia* and *Cystiactis* by Duerden (1895) in the family Aliciidæ. To this, *Thaumactis* (Haddon and Duerden, 1898), *Phymactis* (Haddon, 1898; Carlgren, 1898) and *Phyllodiscus* (Carlgren, 1898) have also been later added, though the heterogeneous character of the Aliciidæ was pointed out by Carlgren (1898). Pax (1914) placed *Bunodeopsis* along with *Alicia*, *Phymactis*, *Cystiactis*, *Rivetia*, *Phlyctenactis* and *Thaumactis* in one family. Stephenson (1921, 1922) referred it

to the Phyllactiidæ, along with *Phyllactis*, *Cradactis* (inclusive of *Saccactis*), *Phymactis* (inclusive of *Rivetia* and *Bunodosoma*), *Cystiactis*, *Lebrunia* and *Thaumactis*.

In his paper on *Boloceroides*, *Bunodeopsis* and their supposed allied genera, Carlgren (1924) enters into a detailed examination of the different characters of all the allied forms and concludes that *Boloceroides* and *Bunodeopsis* are closely allied genera, while the rest of the forms go into two distinct groups: *Alicia*, *Phyllodiscus* and *Lebrunia* forming the Aliciidæ; *Phymactis*, *Rivetia*, *Bunodosoma*, *Cystiactis*, *Phyllactis*, *Saccactis* and *Cradactis* constituting the Phyllactiidæ. The family Myonanthidæ is dropped since, according to him, it is very heterogeneous, *Myonanthus* and probably *Macrodactyla* being Actiniid genera. He creates the new group Boloceroidaria, to include the family Boloceroididæ with *Boloceroides* and *Bunodeopsis* (Carlgren, 1924); and this scheme is completed by a provisional reference of *Nevadne* to a new family Nevadneidæ, under the Boloceroidaria (Carlgren, 1925). Pax in his latest system (1925) accepts the families Boloceroididæ and Phyllactiidæ as defined by Carlgren, but retains the family Myonanthidæ for *Myonanthus*, *Macrodactyla* and *Nevadne*, while in Stephenson's recent monograph (1935) the arrangement suggested by Carlgren is mainly followed.

The presence of a pedal disc without basilar muscles, longitudinal muscles and sometimes spirocysts on the column, the absence or extreme weakness of the sphincter, and the weak muscularity of the mesenteries are cited by Carlgren as the main features of the Boloceroidaria. They retain the longitudinal musculature of the body wall—an essentially Protanthean feature—but the presence of well-developed ciliated tracts on the filaments is an unmistakable Nynanthean feature, and settles once for all their position among the Nynantheæ. After a detailed study of the Boloceroidaria, Carlgren concludes, " In fact, just as there is only one opinion, that *Protanthea* is the most primitive Actiniarian we at present know, I think that *Boloceroides*, having retained the most Protanthean characters, is the most primitive Nynantheæ " (pp. 18–19, 1924). The disparity of opinion that existed on the affinity of the Boloceroidaria seems to have been set at rest as all the available evidences confirm Carlgren's contention.

It was shown in the morphological account how the new anemone described in this paper has all the features characteristic of the Boloceroidaria. This leads to a comparison with the other genera, *Boloceroides*, *Bunodeopsis* and *Nevadne*. It agrees with *Boloceroides* in having a smooth column without vesicles, and in its remarkable powers of asexual reproduction.

These resemblances are so apparent that at first sight it might appear that the anemone is a *Boloceroides*. But *Boloceractis gopalai* differs from the species of *Boloceroides* in the absence of spirocysts on the column, in the possession of distinct siphonoglyphes, and in the fertility of the mesenteries of the last orders. Of these, the last two characters are of considerable importance as they are of generic value, and are used to distinguish certain Endomyarian genera from their close relatives. It is hence necessary to consider *Boloceroides* and *Boloceractis* as distinct genera. There is very little in common between *Bunodeopsis* and *Boloceractis* save the group characters. The presence of distinct siphonoglyphes and fertile imperfect mesenteries in the new genus *Boloceractis* is in sharp contrast to the other members of the Boloceroidaria.

There is some agreement with the Nevadneidæ since the absence of spirocysts and vesicles on the column and the sterility of the primary mesenteries are characteristics of *Nevadne* (Annandale, 1915). The features of *Boloceractis* in agreement with the two families are shown in the following table :—

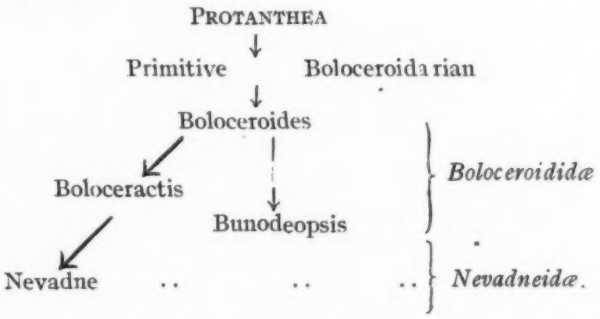
Boloceroididæ.	*Nevadneidæ.*
Column smooth.	Column without spirocysts.
Ectodermal longitudinal muscles through the column.	
Absence of sphincter.	
Deciduous nature of the tentacles.	
Weak muscularity in the mesenteries.	Feeble retractors.
No differentiation into macrocnemes and microcnemes.	No differentiation into macrocnemes and microcnemes.
	Usual sterility of the primary mesenteries.
Column short and not vermiform.	
Prevalence of asexual reproduction.	
Distinct pedal disc without basilar muscles.	Distinct pedal disc without basilar muscles.

This comparison makes it clear that we have to refer *Boloceractis* to the Boloceroididæ as there is great agreement with this family in several important features. In the presence of siphonoglyphes and fertile imperfect mesenteries, the genus shows deviation from the typical Boloceroidid

condition. The arrangement of the tentacles in *Boloceractis* is on the fundamental actinian plan and does not present any peculiarities; but the very curious arrangement of tentacles and later sets of micromesenteries is the most important feature of *Nevadneidæ* which is represented by the solitary species *Nevadne glauca*. Hence we cannot pay so much attention to the features wherein *Boloceractis* and *Nevadne* are in agreement as to consider them as belonging to the same family.

In the evolution of this new genus, the spirocysts on the column wall observed in *Boloceroides* may have been lost; but neither the vesicles as seen in *Bunodeopsis*, nor stinging spots have developed here. The power of regeneration and the sphinctered nature of the tentacles are present as in the Boloceroididæ. Siphonoglyphes have developed and the imperfect mesenteries have become progressively fertile, while there has been a gradual reduction in the reproductive organs in the perfect mesenteries. The *Boloceractis-stage* possibly represents a definite landmark in the evolution of the Nevadneidæ. If so, the development of the vermiform column with stinging spots and the atypical arrangement of tentacles would be later acquisitions of Nevadne.

The evolutionary tendencies indicated by the Boloceroidaria have been discussed by Carlgren (1924) who rightly maintains that the Boloceroidaria have arisen from the Protantheæ as an independent off-shoot in which some of the primitive Protanthean features have been retained. *Boloceroides* must be reckoned as the most primitive genus of the group as it shows the closest resemblance to *Protanthea*. *Bunodeopsis* may have descended from a Boloceroides-like ancestor (*cf.* Carlgren, 1924; Stephenson, 1922). *Boloceractis* must also have descended from a Boloceroides-like ancestor but independent of *Bunodeopsis*. The genus *Nevadne* has perhaps evolved from a form closely related, if not very similar, to *Boloceractis*. The scheme may be represented as follows:—



Summary.

1. The morphology and asexual reproduction of a brackish-water Boloceroidarian from Madras, have been studied.
2. As the anemone differs from other known members of the Boloceroidaria in certain features of generic value, it is referred to a new genus, *Boloceractis*, under the family Boloceroididæ.
3. Generic and specific definitions of *Boloceractis gopalai* are given and the anatomy of the anemone is described.
4. The mesenteries of *Boloceractis gopalai* are not sharply differentiated into macrocnemes and microcnemes; and the gonads are usually borne by the later cycles of imperfect mesenteries, the perfect mesenteries being typically sterile.
5. Asexual reproduction takes place in the anemone by a process of regeneration of the deciduous tentacles, the sphincter ed partitions at the bases of the tentacles playing an important rôle in this process.
6. An account of the habits of the anemone is given and the rôle of the tentacular sphincter of the Boloceroidaria is discussed.
7. The systematic relationships of the genus *Boloceractis* are discussed.

Acknowledgments.

In conclusion, I wish to thank Professor R. Gopala Aiyar for the constant help, encouragement and advice that I have received from him in the preparation of this paper. I am deeply indebted to Professor T. A. Stephenson of the University of Cape Town, for numerous suggestions and helpful criticism. Finally, I have to thank the University of Madras for giving me facilities for this work by awarding me a research studentship.

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EXPLANATION OF FIGURES.

TEXT-FIG. 1.—*Boloceractis gopalai.* A.—Transverse section of the body wall from about the middle of the column; B.—T.S. tentacle. *ect.*—ectoderm; *ect. m. l.*—ectodermal longitudinal muscle layer; *end.*—endoderm; *mes.*—mesoglea; *n.*—nematocysts; *sp.*—spirocysts; and *z.*—zooxanthellæ. × 350.

TEXT-FIG. 2.—Longitudinal section passing through the bases of some of the tentacles of *Boloceractis gopalai.* Partly diagrammatic. *c.w.*—column wall; *l.t.*—lumen of the tentacles; and *p.*—partitions at the bases of the tentacles with the sphincter. × 100.

TEXT-FIG. 3.—Siphonoglyphe of *Boloceractis gopalai* as seen in a transverse section, along with a pair of directives. *d.m.*—directive mesentery; *ect. ph.*—ectoderm of the actinopharynx; *end.*—endoderm; *mes.*—mesoglea; and *si.*—siphonoglyphe. × 350.

TEXT-FIG. 4.—Transverse sections of a perfect mesentery of *Boloceractis gopalai.* In A, the section passes through the lower region where only the median tract is found on the filament. B represents the section through the trifid region of the same mesentery. *cil. tr.*—ciliated tract; *cn. gl. tr.*—cnidoglandular tract; *end. c.*—endoderm of the column; *end. m.*—endoderm of the mesentery; *mes.*—mesoglea; *r.*—retractor; and *ret. tr.*—reticular tract. × 240.

TEXT-FIG. 5.—T.S. of a fertile imperfect mesentery (3rd cycle) of *Boloceractis gopalai.* *cn. gl. tr.*—cnidoglandular tract; *ect.*—ectoderm; *end.*—endoderm of the column; *end. mes.*—endoderm of the mesentery; *mes.*—mesoglea; *ov.*—ovary with developing oocytes; and *z.*—zooxanthellæ. × 300.

PLATE V.

A.—Photograph of adult specimens of *Boloceractis gopalai.* B.—Different stages of regeneration of young individuals from deciduous tentacles. About the natural size.

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A.

B.

Errata.

Vol. IV, No. 6, December 1936.

Page 436, line 18—*for* " Mr. Evans " *read* " Mrs. Evans ".
Page 437, line 17—*for* " Malayas " *read* " Malays ".
Page 441, line 9—*for* " Polyalthia fragnans " *read* " Polyalthia fragrans".
Page 441, line 25—*for* " Mr. Foulke " *read* " Mr. Foulkes ".
Page 443, line 29—*for* " disposal " *read* " dispersal ".

Page 500, line 20—*for* " coveted " *read* " covered ".
Page 508, line 30—*for* " view is not correct " *read* " view is that it is not correct ".
Page 511, line 31—*for* " Veddha " *read* " Veddah ".
Page 512, line 30—*for* " there " *read* " their ".

Table of Measurements.

line 12—*for* " Vaudamet Periza " *read* " Vaudamet, Periyar ".

FERTILISATION AND SEED DEVELOPMENT IN AMARANTACEAE.

By A. C. Joshi
AND
L. B. Kajale.
(*From the Department of Botany, Benares Hindu University.*)

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A number of papers have appeared during recent years on the structure and development of the embryo-sac in the family Amarantacæ, but not much attention has been paid to the development of the embryo. Naithani (1933) and Joshi and Rao (1934) published a few observations on *Digera arvensis*, but these are very meagre and give no adequate idea of the subject. Woodcock (1931) has described the development of the seed in *Amarantus caudatus*, but he deals chiefly with endosperm and perisperm. The present paper therefore is the first detailed account of embryogeny for the family Amarantaceæ.

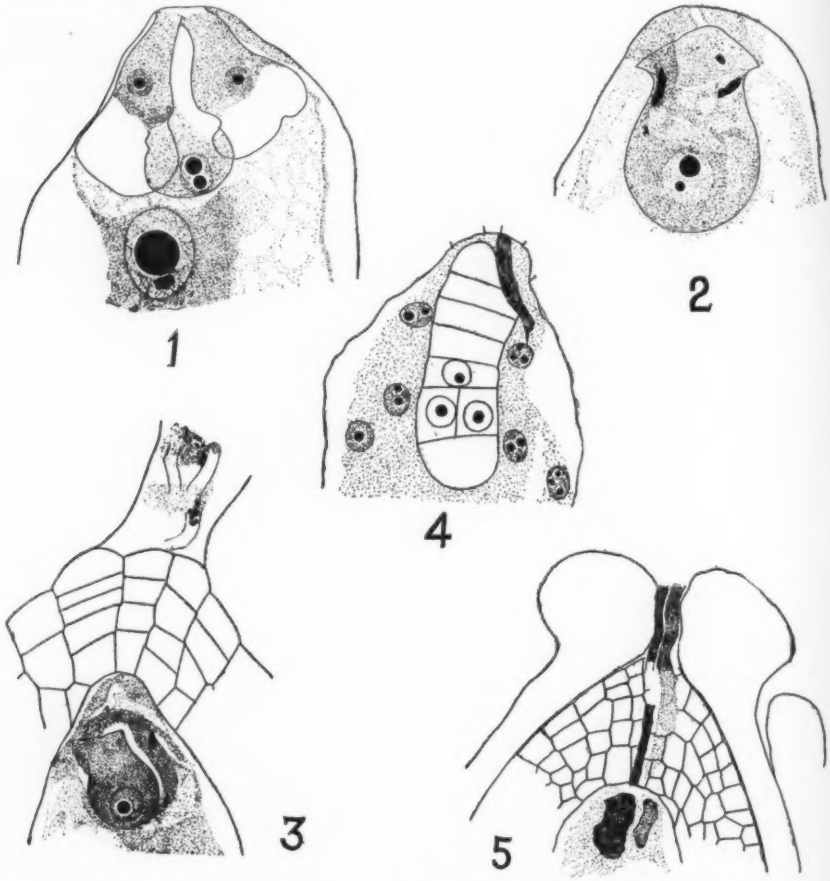
The plants studied include *Alternanthera sessilis* R. Br. and *Digera arvensis* Forsk., some phases of the life-history of which have already been described by Naithani (1933), Joshi and Rao (1934), Puri and Singh (1935), Kajale (1935) and Joshi (1936). During the present investigation, the first plant has been studied in more detail than the second, but the two have been found to agree in all essential points except in the structure of the endosperm.

Fertilisation.

Some stages in fertilisation have already been described in *Digera arvensis* by Joshi and Rao (1934). The present account is based largely on *Alternanthera sessilis*. The pollen grains in this species are 3-nucleate at the time of shedding. On reaching the stigma, these are caught among the glandular hairs and begin to germinate. A pollen tube comes out from each and passes down the hollow style, and then through the hollow interior of the ovary till it reaches the funicle of the ovule. Now it travels along the funicle and enters the micropyle. During its further growth, the pollen tube penetrates through the cells of the nucellus overlying the embryo-sac, punctures the apex of the embryo-sac and ultimately reaches the egg-apparatus. Its entrance frequently leaves the synergids in tact (Fig. 1), as in *Salix* (Chamberlain, 1897), *Silphium* (Merrell, 1900), etc.

The pollen tube reaches the base of the egg before it discharges its contents. The male gametes are 4–6 times as long as broad. They may

be straight or curved at one or both ends. One of the male gametes fuses with the egg and the second with the secondary nucleus (Fig. 1). The male gamete fusing with the egg nucleus was always seen to enter from the chalazal end of the egg. Both the male gametes tend to become spherical as fertilisation progresses.



Figs. 1-4. *Alternanthera sessilis*. Various stages in fertilisation. Fig. 1, micropylar portion of an embryo-sac showing fertilised egg and secondary nucleus and still in tact synergids. Fig. 2, micropylar portion of an embryo-sac showing a fertilised egg and accessory male gametes. Fig. 3, micropylar portion of an ovule showing accessory pollen tubes. Fig. 4, micropylar apex of an embryo-sac showing an accessory pollen tube with male gametes, a small embryo and free endosperm nuclei. Figs. 1-2, × 1450 ; Figs. 3-4, × 900.

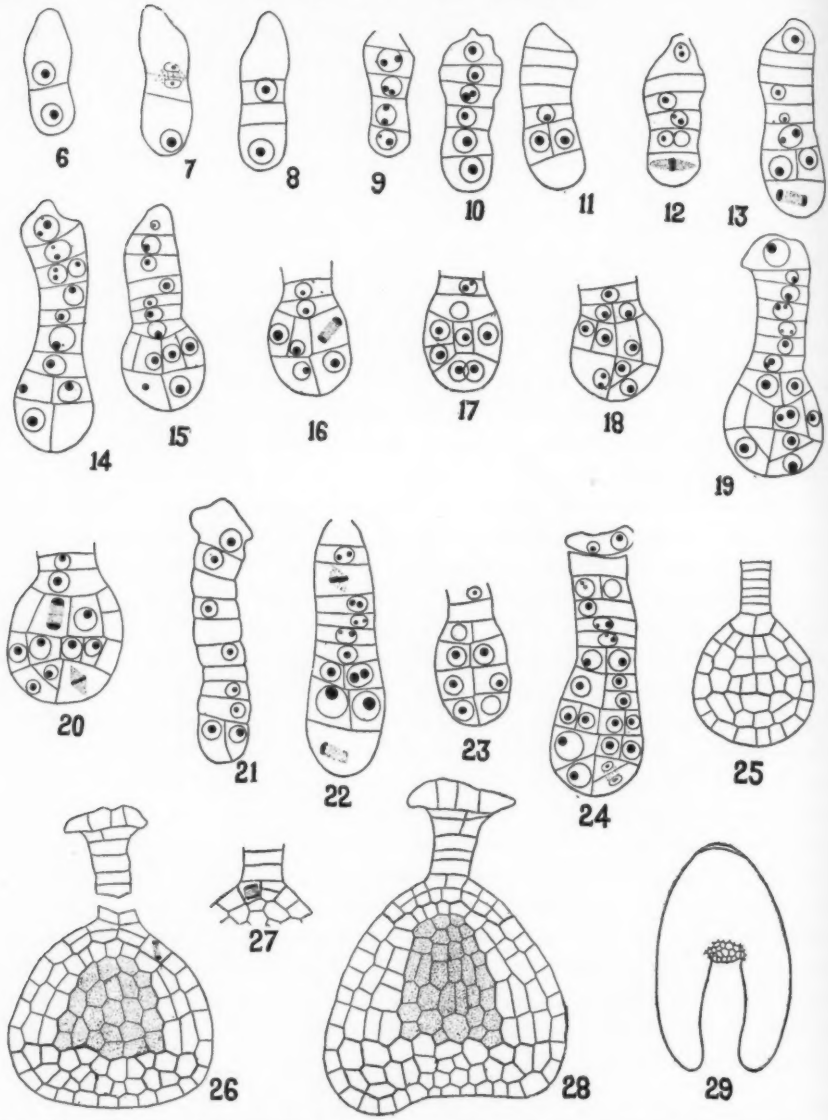
Fig. 5. *Digera arvensis*. Micropylar portion of an ovule showing two pollen tubes. The embryo-sac shows degenerating synergids. × 450.

Two, three or four pollen tubes have been often seen entering an ovule and reaching the embryo-sac, where they discharge their contents even after normal fertilisation has been completed (Figs. 2-3). In one case such an accessory pollen tube with the male gametes inside was seen entering an embryo-sac containing an 8-celled embryo (Fig. 4). Accessory pollen tubes of the same type have also been observed in *Digera arvensis* (Fig. 5). Among other plants the presence of accessory pollen tubes in ovules has been noted in *Elodea canadensis* by Wylie (1904), in *Myricaria germanica* by Frisendahl (1912), *Boerhaavia diffusa* by Maheshwari (1929), etc. Recently Gerassimova (1933) noted as many as six pairs of male gametes inside one ovule of *Crepis capillaris*. In *Elodea canadensis*, Wylie noted cyst formation around the apex of the accessory pollen tubes, but nothing like this has been observed in the plants which form the subject of the present investigation. The accessory pollen tubes in these simply disorganise after some time. The accessory male gametes discharged by these pollen tubes into the embryo-sac also generally share the same fate, but it is possible that very rarely they may unite with a fertilised egg nucleus and thus give rise to polyploid forms. This question will be discussed in a later paper.

Another interesting feature of pollen tube development in *Alternanthera sessilis* is the behaviour of the tube nucleus. Generally in the flowering plants during the development of the pollen tube, it remains quite close to the sperm nuclei, being just ahead of them or just following them. The first condition is more frequent. In the mature pollen grains of *Alternanthera sessilis*, the tube nucleus is a quite prominent spherical structure larger than the sperm nuclei, but in the pollen tubes the tube nucleus was never seen, although quite a large number of them were examined. In some pollen grains the tube nucleus was found in a fragmented and degenerating condition. It is, therefore, probable that the tube nucleus generally degenerates before germination of the pollen grains and never comes out into the pollen tubes. A similar early degeneration of the tube nucleus was also sometimes observed by Frisendahl (1912) in *Myricaria germanica* and by Armand (1912) in *Lobelia erinus*. In the latter species it disappears as soon as the pollen tube has become 20 times as long as the diameter of the pollen grain.

Embryo Development.

The first division in the fertilised egg is transverse and results in the formation of two cells (Figs. 6 and 30). The next division is again transverse and takes place in the micropylar cell of the 2-celled proembryo (Fig. 7). In this way a row of three cells is formed (Fig. 31). More transverse divisions follow, but their exact sequence has not been followed.



FIGS. 6-29. *Alternanthera sessilis*. Various stages in the development of the embryo. In Figs. 6-15, 9-22, 24, 26 and 28-29 complete embryos are shown, in Figs. 16-18, 23 and 25 only their apical part. Fig. 27 shows the differentiation of the hypophysis. Fig. 29 is an outline drawing of an embryo after the differentiation of the cotyledons and the stem tip. The shaded part in Figs. 26 and 28 represents the plerome. For further explanation see Text. Figs. 6-24, ×600; Figs. 25-28, × 390; Fig. 29, ×133.

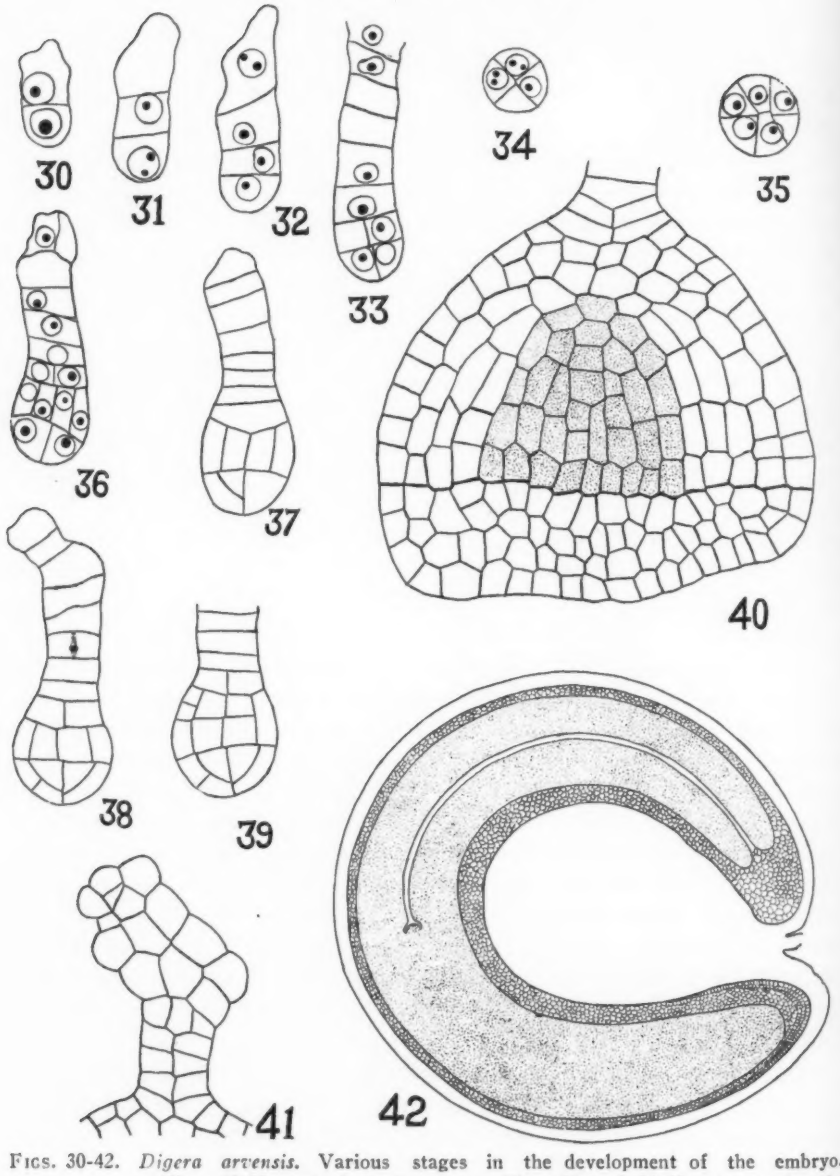
A row of 6–7 cells is generally formed before longitudinal walls appear (Figs. 8–11 and 31). Only exceptionally the proembryo is much longer and consists of 8–11 cells before the appearance of the first longitudinal walls (Fig. 21).

Three apical cells of this proembryo generally take part in the development of the embryo proper. The apical one gives rise to the stem tip and the cotyledons, the sub-apical one to the hypocotyl and the greater part of the root, and the third from the apex forms the hypophysis from which the apex of the embryonic root develops. The embryo development thus corresponds to the chenopodiaceous-type of Schnarf (1929).

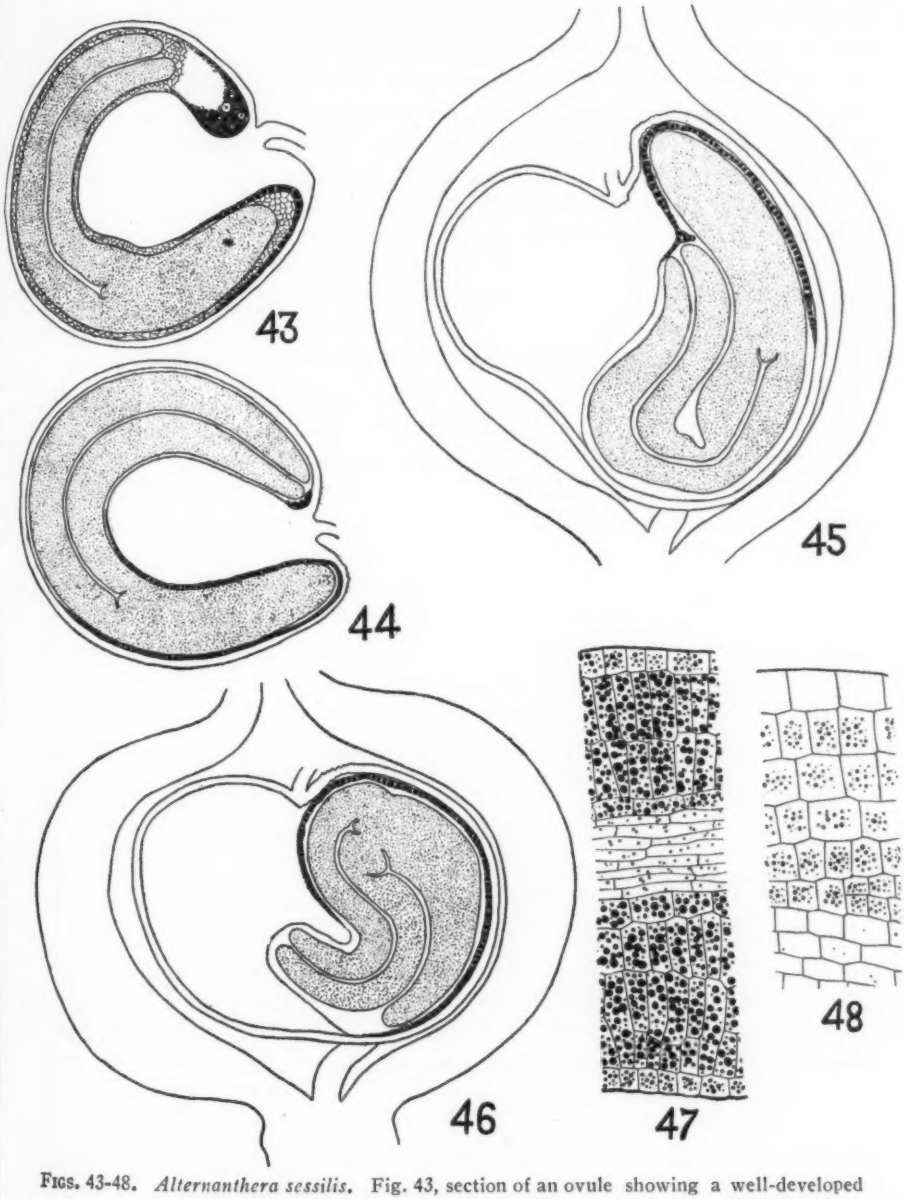
Exceptionally more than three cells of the proembryo may take part in the development of the embryo proper. Thus in Figs. 22, 23 and 24, 4, 4 and 5 cells respectively appear to take such a part, but such cases are rare and have been seen in *Alternanthera sessilis* alone. The accessory cells take part in the development of the hypocotyl and the root region of the embryo only. In this manner, when the embryo is developing from four cells, its development exactly agrees with that of *Chenopodium Bonus-Henricus* studied by Souèges (1920). Normally the sub-apical cell of the proembryo of *Alternanthera* and *Digera* performs the same function as is performed by the second and third cells in *Chenopodium* together.

The sub-apical cell of the proembryo is generally the first to divide by a longitudinal wall (Figs. 11–13). Only very rarely the apical cell may divide before it (Fig. 21). The first longitudinal division in these cells is followed by another in a plane at right angles to the preceding and quadrants are formed (Fig. 34). Two opposite quadrants alone now divide by longitudinal walls. In this way six cells are formed in each tier (Fig. 35). These may be called hexants. Their formation has been clearly seen in *Digera*, but they are also probably organised in *Alternanthera*. Further differentiation of the three histogenic layers in each tier (Figs. 14–28 and 36–40), development of the cotyledons and the stem tip (Figs. 26, 28 and 29), and the differentiation of the root apex from the hypophysis (Figs. 25–28) proceeds in the same manner as described by Souèges (1920) in *Chenopodium Bonus-Henricus*.

The 3–4 cells of the proembryo not taking part in the development of the embryo proper give rise to the suspensor. In *Alternanthera* this remains mostly uni-seriate except at the micropylar end, where the last cell divides lengthwise into 4 or 5 cells. This forms a somewhat peltate structure projecting into the nucellus all round and probably helping in the nutrition of the embryo (Figs. 26 and 28). In *Digera arvensis*, the suspensor is more



FIGS. 30-42. *Digera arvensis*. Various stages in the development of the embryo. Figs. 30-32, proembryonic stages. Figs. 33 and 36-40, stages in the differentiation of the embryo proper. Figs. 34 and 35, transverse sections of young embryos showing the formation of quadrants and hexants. Fig. 41, suspensor. Fig. 42, section of an ovule showing a well-developed embryo, endosperm and perisperm. Figs. 30-41, × 450; Fig. 42, × 48.



FIGS. 43-48. *Alternanthera sessilis*. Fig. 43, section of an ovule showing a well-developed embryo and endosperm. Fig. 44, section of a mature seed. Figs. 45 and 46, sections of fruits showing seeds with abnormal embryos. Figs. 47 and 48, portions of longitudinal sections of a cotyledon and hypocotyl respectively showing the distribution of starch. Figs. 43-46, × 56; Figs. 47-48, × 415.

massive (Fig. 41). It is more than uni-seriate throughout its length and at the micropylar end consists of a mass of cells. Thus it approaches the suspensor of *Amarantus caudatus* figured by Woodcock (1931).

As the embryo develops, it becomes greatly elongated and curved (Figs. 42–44). It forms a horse-shoe-shaped structure in the outer part of the seed. Starch is deposited in all its parts, though at a late stage. It is more abundant in the cotyledons than in the hypocotyl and the root (Figs. 47–48). The individual grains also are comparatively bigger in the cotyledons. Among the three histogenic layers, starch is most abundant in the periblem and least in the plerome (Figs. 47–48).

Endosperm and Perisperm.

The structure of the endosperm of *Alternanthera sessilis* differs from that of *Digera arvensis*. In *Alternanthera*, the endosperm becomes cellular in the larger part of the embryo-sac, but remains free nuclear to the end at the chalazal extremity (Fig. 43). It thus corresponds to the *Melandrium*-type of Rócen (1927). In *Digera*, the endosperm becomes cellular throughout its length (Fig. 42) and corresponds to the *Silene*-type of Rócen (1927). In this species, the endosperm also is not entirely uniform. Two or three layers of cells formed first over the radicle of the embryo consist of more regularly and compactly arranged cells than others formed afterwards (Fig. 42.)

In the mature seed, most of the endosperm is absorbed by the growing embryo. Only a layer or two are left surrounding the radicle and the hypocotyl of the embryo in the form of a cap (Fig. 44), as in *Amarantus caudatus* (Woodcock, 1931). In *Digera arvensis*, these persistent cells are the ones which are more regularly arranged during the early stages. A few starch grains may be seen in these remaining endosperm cells, but they are nearly in a degenerating condition and take little part in storing the reserve carbohydrates of the seed.

The growth of the endosperm and the embryo destroys a part of the nucellus, but its central region remains in tact. Starch grains are abundantly deposited in its cells and it forms the perisperm of the mature seed around which the horse-shce-shaped embryo lies.

Two Abnormal Embryos.

The normal structure of the seed should be clear from the preceding description. Inside the testa, it contains a horse-shoe-shaped embryo surrounding a central mass of perisperm. The endosperm is present only

as a small cap over the radicle and the hypocotyl. Starch is stored in the perisperm and the cells of the embryo. In *Alternanthera sessilis*, however, two cases have been seen in which the form of the embryo and its relation with the perisperm was different. In the first case (Fig. 45), the embryo, instead of being nearly annular, was bent over itself with the cotyledons in an incumbent position. In the second case (Fig. 46), the embryo had a somewhat sigmoid form. Secondly its radicle was not found near the micropyle of the seed, but just at the opposite end. In both these seeds containing abnormal embryos, the perisperm was not surrounded by the embryos, but was situated towards one side of them. The deposition of reserve food materials was also different in these abnormal embryos. Normal embryos in the Amarantaceæ contain starch grains alone, but in the dermatogen of these abnormal embryos the reserve grains were of a different kind, probably aleurone grains. This shows that these abnormal embryos had developed as a result of some physiological disturbance in the metabolism of the ovules in which they were formed.

Summary.

Fertilisation, endosperm and development of the embryo have been studied in *Alternanthera sessilis* and *Digera arvensis*.

Fertilisation is porogamous. Normal double fertilisation takes place. Accessory pollen tubes commonly enter an ovule. Tube nucleus probably degenerates at an early stage, perhaps within the pollen grain itself.

The embryo development corresponds to the chenopodiaceous-type.

The endosperm in *Digera* becomes cellular throughout its length, in *Alternanthera* only in the micropylar half.

The mature seed contains a horse-shoe-shaped embryo surrounding the central perisperm. Endosperm is present only as a cap of one or two layers of cells over the radicle and the hypocotyl. Starch is stored in the embryo and the perisperm. In the embryo, it is more abundant in the cotyledons than in the hypocotyl and the radicle, and more abundant in the periblem than in the dermatogen and the plerome.

Two embryos of a different shape have been noted in *Alternanthera sessilis*. In one case, the embryo was bent over itself. In the second, it had a sigmoid form. The perisperm in both these cases was situated towards one side of the embryo and not surrounded by it.

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A NEW SPECIES OF *ANABAENA* (*ANABAENA AMBIGUA* SP. NOV.).*

By C. Bhashyakarla Rao, M.Sc.

Research Scholar in Botany, Benares Hindu University.

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[Communicated by Prof. Y. Bhâradwâja, M.Sc., Ph.D. (Lond.), F.L.S.]

From a few ponds near the Benares Hindu University grounds a peculiar blue-green alga was collected this year during the latter half of July and the earlier half of August. The plant-body comprises three types of trichomes. Firstly, there are trichomes that are individually surrounded by a mucilaginous sheath (Fig. 1, *B*, and Fig. 2, *B*). Secondly, there are trichomes that are in groups and are enclosed in a common mucilaginous envelope (Fig. 1, *C*, and Fig. 2, *C* and *D*). The thalli comprised by such trichomes are usually attached by one end (Fig. 1, *A-C*) to the grass blades and roots of *Lemna* and *Trapa*. These thalli, which occasionally branch once or twice, are distributed on the host rather sparingly, although cases where they cover it almost completely are not uncommon. Several of these individuals have also been observed to be free-floating after they get detached from the host. Thirdly, there are such free-floating trichomes as are devoid of any mucilage (Fig. 1, *D* and Fig. 2, *A*). As noticed in the living material, the trichomes of the first two types may sometimes emerge out of their sheaths (Fig 1, *B*) one after the other, leaving the latter empty (Fig. 1, *A*), and the emerged trichomes then remain free-floating. This, however, is observed only in the case of such trichomes as have not produced any spores. The empty sheaths are also formed by the disintegration of the trichomes occupying them. They either remain attached to or get detached from the host.

The hyaline mucilaginous sheath enclosing the trichomes is usually firm (only rarely diffluent at places), and possesses a smooth or rough outline and a variable width. It is usually 300–500 μ (rarely upto one mm.) long and 10–50 μ broad. It readily takes up all kinds of stains (*i.e.*, aqueous methyl green, alcoholic safranin, methylene blue, Heidenhain's Hæmatoxyline, etc.), the one enclosing vegetative trichomes staining deeper than that containing sporogenous ones. The reaction with aqueous methyl green and alcoholic safranin shows an affinity of the sheath for basic aniline dyes and indicate its pectic nature [*cf.*, Bhâradwâja[1]]. Chlor-zinc-iodide does not stain the sheath

* From the Department of Botany, Benares Hindu University.

violet even after a long immersion, and this shows the absence of cellulose in the sheath. A dissection of the sheath under the binocular dissecting microscope shows that it is not a hollow cylinder of mucilage but it is a thick micilaginous body in which the trichomes are embedded. The peripheral part of the sheath is of a thicker consistency and stains more deeply than the inner one.

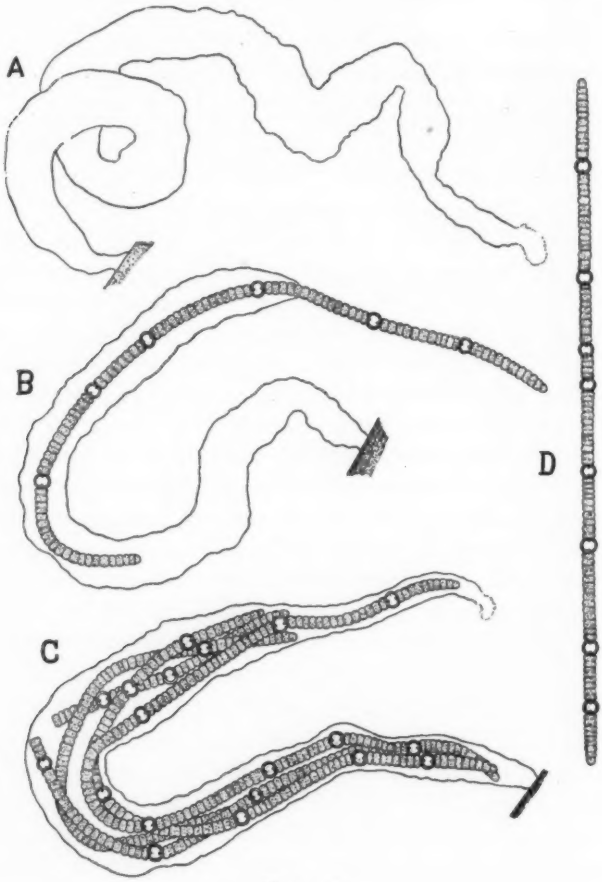

FIG. 1.

FIG. 1. *A*—Empty sheath; *B*—Trichome emerging out of its sheath; *C*—A number of trichomes enveloped by a common mucilaginous sheath; D—Trichome without any mucilaginous envelope. All × 280.

The trichomes are either straight or bent. Those that are in groups within the sheath overlap each other but do not get entangled (Fig. 1, *C*

and Fig. 2, *C* and *D*). They usually taper at the extreme ends, with the end-cells having rounded apices, and measure upto 300 μ in length. Sometimes even longer trichomes have been observed, and one trichome measured as much as 1,400 μ long. The cells are barrel-shaped, being shorter than broad

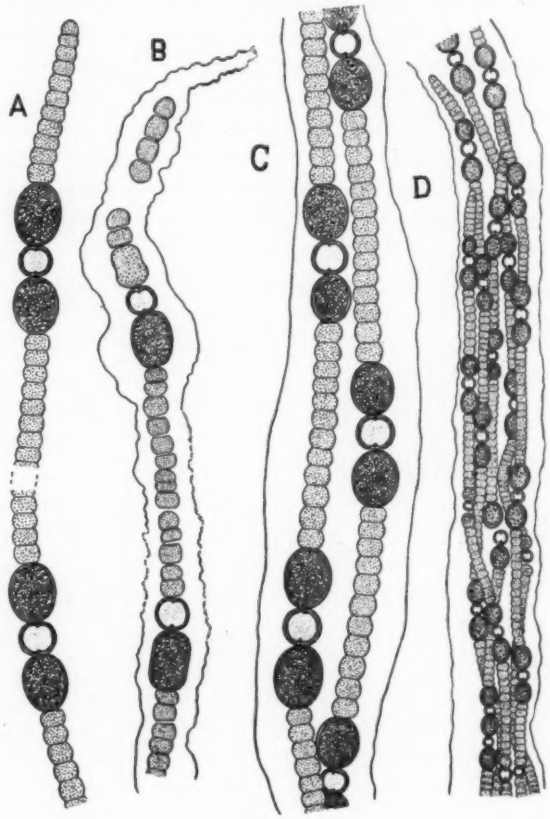

FIG. 2.

FIG. 2. *A*—Sporogenous trichome without any mucilaginous envelope; *B*—Sporogenous trichome with the enveloping sheath; *C* and *D*—Sporogenous trichomes enclosed in a common mucilaginous sheath. *A*—*C* × 580; *D* × 280.

or as long as broad, with deep constrictions at the cross-walls. They are 4·9–6·6 μ broad and 3·3–5 μ long. The dissepiments are not very clear but they become distinct on treatment with an alcoholic solution of iodine. The cell-contents are deep blue-green and coarsely granular. The walls and the contents of the cells show practically the same reactions to various stains

and reagents as have been described by Bhâradwâja[1] for *Cylindrospermum muscicola* var. *kashmirensis*.

The heterocysts, which are usually upto fifteen in number, are distributed at intervals throughout the length of the trichome. In one case, however, twenty-two heterocysts were observed. They are more or less spherical, sometimes with slightly flattened ends. They have homogeneous contents of a pale blue-greeen colour, and are usually broader than the vegetative cells, measuring 6·4–9 μ (rarely upto 10 μ) in diameter. The observations made with regard to heterocysts by Fritsch[5] in *Anabæna* and by Bhâradwâja[2] in *Spelæopogon Kashyapi*, and the reactions of their walls and contents to various stains and reagents described by Bhâradwâja[1] for *Cylindrospermum muscicola* var. *kashmirensis*, *Aulosira Fritschii* and *A. prolifica* are in close agreement with those of the present alga.

The spores are formed in trichomes that are enclosed in a sheath (Fig. 2, *B-D*) as well as in those that are free (Fig. 2, *A*). There is generally a spore on either side of a heterocyst, but cases where a spore is formed only on one side of a heterocyst are also met with. The contents of the spores are yellowish-green, with numerous large granules occupying the whole protoplast. The granules are spherical or sub-spherical, and are of different sizes. The reactions of the envelopes and the contents of the spores to various stains and reagents described by Bhâradwâja[1] for *Cylindrospermum muscicola* var. *kashmirensis* and *Aulosira Fritschii* are in agreement with those of the alga described here. The spores are ellipsoidal, sometimes cylindrical with rounded or occasionally with slightly flattened ends. They are much larger than the heterocysts, the mature ones being 8·4–10·9 μ broad and 13·3–16·2 μ long.

The alga, as already pointed out, has three types of structure, *viz.*, (1) a number of trichomes enclosed in a common sheath, (2) individual trichomes enveloped by a sheath and (3) ordinary trichomes without any sheath. The first two types readily recall the condition present in *Anabæna vaginicola* described by Fritsch[7] from South Africa and *Anabænothrix epiphytica*, an *Anabæna*-like alga recently described by Randhawa.[10] The first type is also present in *Anabænothrix cylindrica* Randhawa,† another alga described by the same author.[10] Randhawa has created this new genus *Anabænothrix*, as distinct from *Anabæna*, merely on account of a common sheath enclosing

† Randhawa keeps the words "Randhawa and Ghose" after the names of these plants, but since the paper describing these algæ is published on his name alone, it is not clear how the name of any junior author can be attached to the authorship of the proposed new species, when the latter has no share in the authorship of the paper itself. Moreover, no mention of the actual locality in the Punjab from where these algæ were collected is made by the author.

a number of trichomes. He has also included in it *Anabæna vaginicola* Fritsch under the name of *Anabænothrix vaginicola* (Fritsch) nov. comb.

In the description of *Anabænothrix cylindrica* there is no mention of the presence of a single trichome enclosed in a sheath, while in *Anabæna vaginicola* Fritsch, the presence of individual trichomes surrounded by a sheath is recorded, though it is a rare feature. About *Anabænothrix epiphytica* the author simply mentions that it has " numerous trichomes enclosed in a single sheath or a single one in a single sheath ". This statement, however, does not give any idea of the percentage of either of the two types of structure present. The Benares alga, on the other hand, possesses about 20% of such cases as have individual trichomes surrounded by a sheath, and this feature is also present in *Anabæna Halbfassi* Bachmann, *A. inæqualis* (Kütz.) Born. et Flah., *A. Poulseniana* Boye-Pet. (Geitler[8], Figs. 575, 578 and 579 respectively) and *Anabæna laxa* (Rab.) A. Br. (Tilden[11], Pl. IX, Fig. 18) and also in one other form of *Anabæna* described by Fritsch.[5] Again nearly 40% of the individuals in the Benares form consist of trichomes devoid of any sheath, and this is a typical feature in the genus *Anabæna*. Such free trichomes have not been recorded in any of the three forms comprising the genus *Anabænothrix*, which the present alga, however resembles in having a number of trichomes enclosed in a common sheath, though only in about 40% cases.

The Benares plant thus combines the characters of *Anabæna* and *Anabænothrix*, and since in the majority of the cases the plant-body resembles the species of *Anabæna*, the writer is inclined to refer it to this genus for the present. Moreover, the establishment of the genus *Anabænothrix* based merely on the presence of a sheath enclosing one or more *Anabæna*-like trichomes appears open to question. The validity of the genus *Schizothrix* Kütz., which was established on a similar basis, is also questionable, as this genus does not possess any distinctive characters and is likely to be confused in certain cases with *Lyngbya*. In describing *Schizothrix*, West and Fritsch[12] (p. 474) write that it has " trichomes relatively few (sometimes only one) within each sheath". In *Schizothrix Viguieri* Frémy, *S. Bioreti* Frémy, *S. lutea* Frémy (Frémy,[4] pp. 94, 99 and 108 respectively), *S. antarctica* Fritsch (Fritsch,[6] p. 11), *S. ecalcarea* Gardner, *S. Willei* (Gardner) Geitler, *S. Gomontii* Weber van Bosse, *S. mellea* Gardner (Geitler,[8] pp. 1094, 1098, 1100 and 1115 respectively) and others the presence of individual trichomes enclosed by a sheath is recorded, while *Schizothrix perforans* (Ercegović) Geitler (Geitler,[9] p. 446), *S. longiarticulata* Gardner, *S. Arnotti* Frémy, and *S. Heufleri* Grün. var. *incrustans* Ercegović (Geitler,[8] pp. 1087, 1104 and 1107 respectively) possess mainly this type of trichomes. In all these

cases such trichomes are hardly different from those of the species of *Lyngbya*. About *Schizothrix perforans* (Ercegović) Geitl., Geitler[6] (p. 1079) writes, " Kann daher mit *Lyngbya* verwechselt werden" and also " Die Art kann allenfalls als *Lyngbya* angesehen werden". There is also in many cases, no distinctive differences in the strata formed by these two genera in nature.

The filaments of *Lyngbya* themselves are likely to be confused, in certain cases, with those of the species of *Phormidium* (*e.g.*, *Phormidium mucosum* Gardner, Geitler,[8] Fig. 646 *b*) in which the sheath is persistent. In such cases, however, the only difference between these two genera lies in the nature of their strata, as West and Fritsch[12] (p. 473) have pointed out that " The determination of species without knowing the characteristics of the stratum is scarcely to be advocated." But this difference in habit also in these imperfectly known blue-green algæ may, after further experimental work, turn out to be of no great taxonomic importance. As an illustration the writer would mention *Nostochopsis radians* Bhâradwâja, which in spite of its having quite a different habit from that of the other known species of the genus, has not been raised to a generic rank, and would further quote what the author[3] of this form says about *Myxoderma*: "The retention of *Myxoderma*, which differs only in habit from *Nostochopsis*, also appears open to question."

From what has been stated above, the writer is of the opinion that these three genera—*Schizothrix*, *Lyngbya* and *Phormidium*—should be subjected to a careful revision. In this connection it may be suggested that all such species of *Schizothrix*, which possess mainly *Lyngbya*-like trichomes should be accommodated in the genus *Lyngbya* in a seperate section created for the purpose. Again, the species of *Phormidium* in which the trichomes possess a persistent sheath should likewise be included in the genus *Lyngbya* under a different section.[1] Further, all such species of *Phormidium* in which the sheath of the filaments is mostly in a dissolved or diffluent state should be accommodated in a separate section of the genus *Oscillatoria*.[2] In the same way the forms belonging to the genus *Anabænothrix* should be included in the genus *Anabæna* Bory under a separate section '*Anabænothrix*' to accommodate such *Anabæna*-like forms as possess one or more trichomes enclosed in a common mucilaginous sheath.

The Benares form approaches *Anabæna sphærica* Born. et Flah., which it resembles in the barrel-shaped cells, the spherical heterocysts, and in the formation of a single spore on each side of a heterocyst; but it differs from the same in the bigger heterocysts and smaller ellipsoidal spores and also in the frequent presence of trichomes enclosed in a sheath.

The present alga also shows a resemblance with *Anabæna vaginicola* Fritsch in having spores adjacent to heterocysts, besides having one or more trichomes surrounded by a sheath. But it differs from this South African form in the usually firm nature of the sheath, in possessing broader trichomes, smaller barrel-shaped cells and spherical heterocysts, and also in having spores that are broader and shorter and situated singly on either side of a heterocyst.

The Benares alga also differs from *Anabænothrix cylindrica* in having broader barrel-shaped cells, smaller heterocysts and ellipsoidal spores that are broader and shorter, and are formed singly on either side of a heterocyst. It further differs from *Anabænothrix epiphytica* in having broader barrel-shaped cells and broader and shorter spores, that are formed singly on either side of a heterocyst.§ The Benares form may therefore be considered a new species of *Anabæna* and may be named *A. ambigua* sp. nov.

Diagnosis.

Trichomes free or completely enclosed in a mucilaginous envelope or sheath, in the latter case occurring either singly or in groups; trichomes without sheaths free-floating; ensheathed trichomes free-floating or attached to the host by one end, generally occurring singly but occasionally in dense clusters. Sheath usually firm, hyaline, with smooth or rough outline; usually 300–500 μ (rarely upto one mm.) long, 10–50 μ broad. Trichomes straight or bent, usually slightly tapering at the ends, with end-cells having rounded apices; usually 250–300 μ long, sometimes longer. Cells barrel-shaped, with deep constrictions at the joints; septa indistinct; cell-contents deep blue-green and coarsely granular; 4·9–6·6 μ broad, 3·3–5 μ long. Heterocysts at intervals, almost spherical, sometimes with slightly flattened ends, usually broader than the cells, 6·4–9 μ (rarely upto 10 μ) in diameter. Spores formed in free trichomes as well as in those enclosed in sheath, one on each side of a heterocyst, usually ellipsoidal, sometimes cylindrical with rounded or occasionally flattened ends, with thick hyaline exospore and thin transparent endospore; 8·4–10·9 μ broad, 13·3–16·2 μ long.

Habitat :—Free-floating or attached to grass-blades and roots of *Lemna* and *Trapa* in some ponds near the Benares Hindu University. (July and August, 1936.)

The writer wishes, in conclusion, to express his great indebtedness to Professor Y. Bhâradwâja, for his kind guidance and criticism.

§ Since the author of *Anabænothrix epiphytica* has not given the dimensions of the heterocysts, it is not possible to compare them with those of the Benares form.

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A NOTE ON THE DISTINCTIVE CHARACTERS OF THE INDIAN SPECIES OF *CYLINDROPHIS* WAGLER.

By Beni Charan Mahendra.

(From the Department of Zoology, St. John's College, Agra.)

Received March 3, 1937.

(Communicated by Prof. C. R. Narayan Rao, M.A.)

In a recent paper (Mahendra, 1936), I showed that the three species of the genus *Cylindrophis* Wagler, found within the limits of the Indian Empire, do not differ from each other in the generally accepted specific features. I also published a new key for the genus, proposed by Dr. Malcolm Smith.

In a letter, dated February 3, 1937, Dr. Smith draws my attention to the fact that the key, as published, is unworkable owing to an unfortunate error that has crept into it through oversight. At his suggestion, I hasten to present in its place the following revised key, drawn up by Dr. Smith after an examination of more material. It is found that the proportions of the prefrontals are too variable to be relied on. The colour pattern, however, is constant and distinctive.

I. Breadth of frontal equal to or greater than half the distance between the centres of the eyes; rostral narrow, as high as broad.

  (*a*) Back with or without light cross-bars. *C. rufus.*

  (*b*) Back with two white longitudinal stripes. *C. lineatus.*

II. Breadth of frontal less than half the distance between the centres of the eyes; rostral broader than high.

  Back with a black network enclosing large reddish-brown spots. .. .. *C. maculatus.*

The other members of the genus, which are not found within the Indian Empire, are more easily distinguished, and De Rooij's Key for them is quite sufficient.

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THE ZYGNEMACEÆ OF KASHMIR—I.

By Jogendra Nath Misra, B.Sc.

(*From the Department of Botany, Benares Hindu University.*)

Received November 4, 1936.

[Communicated by Prof. Y. Bhâradwâja, M.Sc., Ph.D. (Lond.), F.L.S.]

Although the Jammu and Kashmir State, the world-famous summer resort in India and popularly known as the 'Switzerland of Asia', possesses extensive marshy places, inundated crop-fields and innumerable lakes and watercourses to harbour inexhaustible treasures of freshwater algæ, there is, at present, practically no record of these plants. The Department of Botany of the Benares Hindu University has therefore planned to describe the algal flora of this most beautiful and unexplored part of the country in a series of papers. The present account deals with some of the Zygnemaceæ, forming a small portion of a large collection of algae made by Prof. Y. Bhâradwâja in 1931, during the months of May and June. In all nineteen forms have been recorded, and out of these four species, four varieties and six forms are new.

Systematic Enumeration of the Species Observed.

Genus *Zygnema* Agardh.

1. *Zygnema indica* sp. nov. (Fig. 1, *A*).

Conjugation only scalariform. Zygospore formation in the conjugation-canal, which becomes bulged out. Zygospores ellipsoidal, with long axis at right angles to the conjugating cells; exospore thin and smooth; mesospore slightly thick, smooth and blue.

Lat. cell., 36–41 μ; long. cell., 56–66 μ; crass. zygosp., 43–46 μ; long. zygosp., 50–60 μ.

Habitat.—In a road-side pond, Srinagar.

According to the key given by Czurda (*Süsswasser-flora Mitteleuropas*, Heft 9, 1932, p. 99), the alga belongs to the Section 'Pectinata' on account of the zygospores being placed in the conjugation-canal. It approaches *Zygnema cyanosporum* Cleve and *Z. majus* Czurda on account of the blue and smooth mesospore, but it differs from both these species in possessing much broader cells and ellipsoidal zygospores. It further contrasts with the latter species in having thinner mesospore.

2. *Zygnema melanosporum* Lagerh. Czurda, *op. cit.*, 1932, p. 116.

Forma *dalense* form. nov. (Fig. 1, *B*).

Lat. cell., 30–32 μ ; long. cell., 99 μ ; crass. zygosp., 30–31 μ ; long. zygosp., 40–46 μ.

Habitat.—In Dal Lake, Srinagar.

The alga agrees with the description given for the type, but it differs in possessing broader filaments and thin mesospore.*

3. *Zygnema sphærica* sp. nov. (Fig. 1, *C*).

Conjugation scalariform. Zygospore formation in the gametangia; fructifying cells not swollen. Zygospores spherical or almost spherical. Exospore thin and smooth; mesospore thin, smooth and blue.

Lat. cell., 26 μ ; long. cell., 53–59 μ ; crass. zygosp., 28–32 μ ; long. zygosp., 29–33 μ .

Habitat.—In a side-way pond, Shalimar Bagh, Srinagar.

The alga falls in the Section Leiosperma on account of the zygospores being placed in the gametangia. It comes close to *Zygnema melanosporum* Lagerheim on account of the thickness of the filaments, the mesospore being smooth and blue, and the fructifying cells being unswollen. But it differs remarkably in possessing spherical zygospores with thin mesospore.*

Forma.

Lat. cell., 30–33 μ ; long. cell., 50–53 μ ; crass. zygosp., 30–31 μ ; long. zygosp., 30–32 μ.

Habitat.—At the bottom of a pool, Ramsu.

The form differs from the type on account of broader filaments.

Forma *longiarticulata* form. nov.

Lat. cell., 31 μ ; long. cell., 70 μ ; crass. zygosp., 30-33 μ.

Habitat.—In a road-side pond, Srinagar.

This form is characterised by much longer cells.

Forma *crassa* form. nov.

Lat. cell., 36–40 μ ; long. cell., 53 μ ; crass. zygosp., 36 μ ; long. zygosp., 40 μ.

Habitat.—In a road-side pond, Srinagar.

This form differs from the type on account of much broader cells and bigger zygospores.

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* The mesospore of the zygospore in the Kashmir alga is smooth as has been described for *Zygnema melanosporum* Lagerheim by Czurda (*op. cit.*, 1932, p. 116). Transeau and his collaborators (Transeau, Tiffany, Taft and Li, "New species of Zygnemataceæ," *Trans. Micros. Soc.*, Vol. LIII, No. 3, 1934), however, write, on the basis of examination of the specimen from the original Legerheim collection, that the mesospore is 'finely punctate'

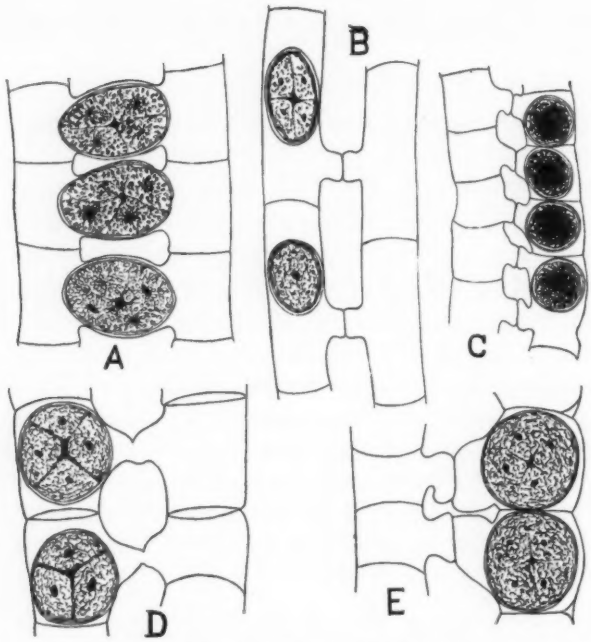

FIG. 1. *A*—Portion of the conjugating filaments of *Zygnema indica* sp. nov. with zygospores; *B*—the same of *Zygnema melanosporum* Lagerh. forma *dalense* form. nov.; *C*—the same of *Zygnema sphærica* sp. nov.; *D*—the same of *Zygnema kashmirensis* sp. nov.; *E*—the same of *Zygnema atrocæruleum* W. et G. S. Weet var. *crassa* var. nov. All × 280.

4. *Zygnema kashmirensis* sp. nov. (Fig. 1, *D*).

Conjugation scalariform. Zygospore formation in the gametangia; fructifying cells unswollen. Zygospores almost spherical. Exospore thin and smooth; mesospore thin, smooth and brown.

Lat. cell., 43–50 μ; long. cell., 43–56 μ; crass. zygosp., 52–53 μ.

Habitat.—In a road-side pond, Srinagar.

This alga comes under Section Leiosperma, and can only be compared with *Zygnema leiospermum* de Bary on account of the spherical form of the zygospores and the smooth and brown mesospore, but it differs in possessing much broader cells and bigger zygospores with thin mesospore.

5. *Zygnema atrocæruleum* W. et G. S. West. Czurda, *op. cit.*, 1932, p. 124.

Var. *crassa* var. nov. (Fig. 1, *E*).

Conjugation scalariform. Zygospore formation in the gametangia;

fructifying cells unswollen. Zygospores spherical to ellipsoidal; exospore thin and smooth; mesospore thin, smooth and blue.

Lat. cell., 30–40 μ; long. cell., 59–66 μ; crass. zygosp., 40–50 μ; long. zygosp., 53–66 μ.

Habitat.—In a road-side pond, Srinagar.

The alga agrees with the type in the scalariform conjugation, the formation of spherical zygospores, in the swollen gametangia and in the presence of smooth and blue mesospore but differs from the same in having broader filaments and bigger zygospores that are also sometimes ellipsoidal.

Genus *Spirogyra* Link.

6. *Spirogyra Spreeiana* Rabenhorst. Czurda, *op. cit.*, 1932, Fig. 147.

Lat. cell., 20 μ; long. cell., 132–181 μ; crass. zygosp., 40–41 μ; long. zygosp., 73–76 μ.

Habitat.—In a rain-water pond, Matton.

Var. *kashmirensis* var. nov. (Fig. 2, *A* and *B*).

Vegetative cells 5–6 times as long as broad; end-walls replicate; chloroplast single. Conjugation lateral; fructifying cells swollen. Zygospores ellipsoidal; exospore thin, smooth and colourless; mesospore thin, smooth and yellow; endospore indistinct.

Lat. cell., 13–16 μ; long. cell., 66–69 μ; crass. zygosp., 26–33 μ; long. zygosp., 50–73 μ.

Habitat.—In a side-way pond, Shalimar Bagh, Srinagar.

The variety agrees with the type in the possession of replicate walls, single chloroplast, swollen fructifying cells and ellipsoidal zygospores, but differs in the conjugation being always lateral, narrower cells and thin mesospore.

7. *Spirogyra velata* Nordstedt. Czurda, *op. cit.*, 1932, Fig. 166 (Fig. 2, *C*).

Lat. cell., 30–36 μ; long. cell., 116–120 μ; crass. zygosp., 33–36 μ; long. zygosp., 50–69 μ.

Habitat.—In Dal Lake, Srinagar.

8. *Spirogyra subsalsa* Kützing. Czurda, *op. cit.*, 1932, p. 167, Fig. 171.

Forma (Fig. 2, *D*).

Lat cell., 25–33 μ; long. cell., 73–132 μ; crass. zygosp., 26–33 μ; long. zygosp., 36–46 μ.

Habitat.—In a road-side pond, Ramsu.

The form differs from the type in having always one chloroplast and slightly bigger zygospores with a thin mesospore.

9. *Spirogyra parvula* (Trans.) Czurda. Czurda, *op. cit.*, 1932, Fig. 174.

Lat. cell., 23–25 μ; long. cell., 152–172 μ; crass. zygosp., 30–33 μ; long. zygosp., 43–52 μ.

Habitat.—In a tank at Uday Lodge, Srinagar, the residence of the late Rai Bahadur Pandit Uday Chand, M.A.

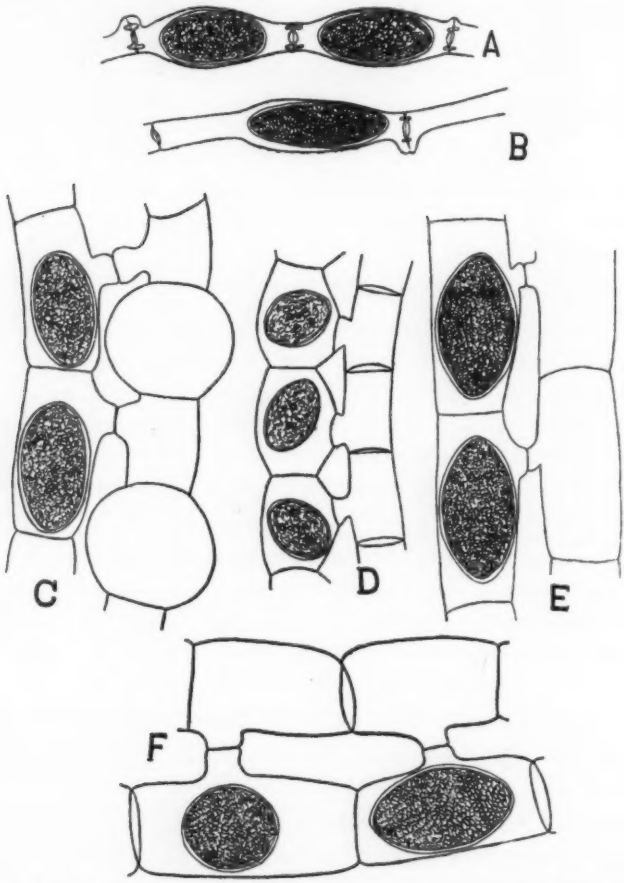

FIG. 2. *A* and *B*—Portion of the conjugating filaments of *Spirogyra Spreciana* Rabenhorst var. *kashmirensis* var. nov. with zygospores; *C*—the same of *Spirogyra velata* Nordstedt; *D*—the same of *Spirogyra subsalsa* Kützing forma; *E*—the same of *Spirogyra decimina* (Mull.) Czurda forma *longispora* form. nov.; *F*—the same of *Spirogyra lacustris* Czurda forma. All × 280.

10. *Spirogyra decimina* (Mull.) Czurda. Czurda, *op. cit.*, 1932, Fig. 181.

Forma *longispora* form. nov. (Fig. 2, *E*).

Lat. cell., 30–36 μ ; long. cell., 89–231 μ ; crass. zygosp., 33–43 μ ; long. zygosp., 59–82 μ.

Habitat.—In a pond 22 miles off Srinagar towards Anantnag.

The form differs from the type in the slightly broader filaments, shorter conjugation-canals and longer zygospores without any rib-line.

11. *Spirogyra lacustris* Czurda. Czurda, *op. cit.*, 1932, Fig. 182.

Forma (Fig. 2, *F*).

Lat. cell., 42–48 μ ; long. cell., 142–181 μ ; crass. zygosp., 45–50 μ ; long. zygosp., 79–82 μ.

Habitat.—In a rain-water pond, Matton.

The form differs from the type on account of the broader filaments and the absence of any rib-line on the zygospores which may occasionally be spherical.

Forma *tenuis* form. nov.

Lat. cell., 33–37 μ ; long. cell., 147 μ ; crass. zygosp., 30–33 μ ; long. zygosp., 50–66 μ.

Habitat.—In a pond at Achhabal.

The form differs from the type on account of narrower cells and smaller zygospores without any rib-line.

12. *Spirogyra Czurdæ* sp. nov. (Fig. 3, *B* and *C*).

Vegetative cells cylindrical, five times as long as broad ; end-walls plane ; chloroplast single. Conjugation scalariform ; fructifying cells not swollen. Zygospores spherical ; exospore thin, smooth and colourless ; mesospore brown, thick, lamellate with broad scrobiculations ; endospore indistinct.

Lat. cell., 26–28 μ ; long. cell., 148–150 μ ; crass. zygosp., 33–34 μ.

Habitat.—In a pond, Matton.

The presence of many-layered and a scrobiculated mesospore is a unique feature in this alga. It can be compared with *Spirogyra dædalea* Lagerheim and *Spirogyra esthonica* Skuja on account of the plane end-walls, scalariform conjugation and the thick and sculptured mesospore. It, however, differs from both these species in having narrower filaments and much smaller spherical zygospores provided with a stratified and scrobiculated mesospore.

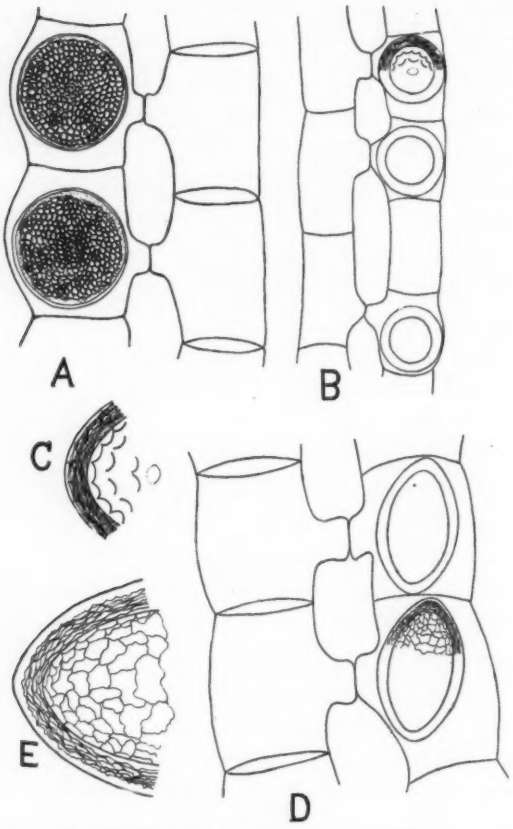

FIG. 3. *A*—Conjugating filaments of *Spirogyra ternata* Ripart var. *sphaerica* var. nov.; *B*—Conjugating filaments and *C*—surface view of the mesospore of the zygospore of *Spirogyra Czurdæ* sp. nov.; *D*—conjugating filaments and *E*—surface view of the mesospore of the zygospore of *Spirogyra Malmeana* Hirn var. *mattonse* var. nov. *A*, *B* and *D* × 280; *C* and *E* × 575.

13. *Spirogyra ternata* Ripart. Czurda, *op. cit.*, 1932, Fig. 197.

Var. *sphærica* var. nov. (Fig. 3, *A*).

Vegetative cells cylindrical, twice as long as broad; end-walls plane; chloroplasts 3–4 (usually 4). Conjugation scalariform; fructifying cells swollen. Zygospores spherical. Exospore thin, smooth and colourless; mesospore thick, smooth and bluish; endospore indistinct.

Lat. cell., 60–63 μ; long. cell, 82–142 μ; crass. zygosp., 59–63 μ.

Habitat.—In a road-side pond, Srinagar.

The alga agrees with the type in the thickness of the filaments and the thick mesospore besides having scalariform conjugation and swollen fructifying cells, but it differs in the chloroplasts being normally four, in the zygospores being always spherical and not ellipsoidal.

14. *Spirogyra Malmeana* Hirn. Czurda, *op. cit.*, 1932, Fig. 216.

Var. *mattonse* var. nov. (Fig. 3, *D* and *E*).

Vegetative cells cylindrical, 3–4 times as long as broad ; end-walls plane; chloroplasts two. Conjugation scalariform ; fructifying cells unswollen. Zygospores ellipsoidal ; exospore thin, smooth and colourless ; mesospore thick, yellowish-brown and reticulately sculptured ; endospore indistinct.

Lat. cell., 79–81 μ ; long. cell., 224–264 μ ; crass. zygosp., 46 μ ; long. zygosp., 69–71 μ.

Habitat.—In a pond at Matton.

The variety agrees with the type in the thickness of the filaments, scalariform conjugation, unswollen fructifying cells and ellipsoidal zygospores with a thick and yellowish-brown reticulately sculptured mesospore but differs in the cells having two chloroplasts and smaller zygospores with a more regular sculpture on the mesospore.

In conclusion, the writer wishes to express his great indebtedness to Professor Y. Bhâradwâja, for his kind guidance and criticism throughout the course of this investigation.

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ON THE CLASSIFICATION AND SCHIZOGONIC CYCLE OF A BLOOD PARASITE OF THE INDIAN LIZARD *CALOTES VERSICOLOR* DAUD. SUBSPECIES *MAJOR* BLYTH.

By Col. I. Froilano de Mello

and

Caetano Corrêa de Meyrelles.

(*Nova Gôa.*)

Received March 9, 1937.

Introduction.

Many specimens of *Calotes* have been during years examined by the senior author of this paper, without being able to detect in their blood any parasite at all.

The present lizard was caught by my pupil C. C. Meyrelles at Nova Gôa and in its blood smears, stained by Leishmann's stain and by May-Grunwald-Giemsa, a parasite was seen whose further study was completed through iron-hæmatoxylin coloration and sections of liver, lungs and intestine.

In the description of this parasite we will record *pari passu* our findings, almost chronologically, in order to deduce logically the conclusions to which we have arrived, trying then in a final discussion to classify the genus and the species of the parasite.

Morphology of the parasite in peripheral blood smears stained at Romanowsky.—In smears of peripheral blood stained at Romanowsky (Leishmann's stain, May-Grunwald-Giemsa) the parasite has the appearance of a *Lankesterella*: the same minute size compared with the size of the red corpuscles, the same elongated, rather fusiform aspect, very seldom roundish or oval. The infection is rather heavy; but the parasite has no visible influence on the red cell, which is not hypertrophied, neither has its nucleus displaced to the periphery. The parasite has a strong membrane, reddish violet, this coloration being deeper with May-Grunwald-Giemsa. The membrane is not uniform: in some places it has some violet reddish dots, in other places some fibres running irregularly from one border to the other, often the whole substance tinges in a more or less deep rose tone more pronounced in some parts of the body,

which otherwise is unstained and appears as a white interruption in the hæmoglobinic mass of the red cell. Among the contents of its protoplasm, there are three zones stained in light blue which will be described now. One central zone, often large and occupying the whole breadth of the parasite, often reduced to a point. At a little distance, another, very regular, oval body, stained in the same light blue and surrounded by a clear halo, suggesting the idea of a membrane. The situation of this body varies greatly, being sometimes entirely polar, sometimes very close to the central zone. Finally, on the opposite pole we find a light blue zone, which begins at the extremity of the pole and is lost in the protoplasm to a certain distance, but does not show any definite outline. This zone may sometimes miss.

In our coloured plate [Pl. VI (Fig. 1)] the different aspects of the parasite are shown. We were not able to interpret in Romanowsky-stained smears the structure of the parasite but we did it subsequently by other methods.

Blood smears stained by iron-hæmatoxylin of Heidenhain.—A special precaution must be taken here in differentiating the hæmatoxylin, as the parasite discolours very rapidly. The best differentiation was with chlohydric alcohol at 1/4 per cent. acting during some seconds only. The solution of alun as differentiator did not give good results. The protoplasm was sometimes stained with eosin or light green, which were specially useful for studying the nuclear centriole.

The parasite now is seen under a very uniform and monomorphic appearance : a strong membrane limiting an unstained body where three definite organelles are noticed : (*a*) a central one, strongly siderophyl, occupying or not the whole breadth of the parasite and which we interpret as nucleus ; (*b*) another roundish body attached to or to a certain distance from the nucleus, staining more lightly and showing seldom a minute, more siderophyl granule, from which presumably it is a dependence. We will call it *paranuclear body* (Fig. 2, *pn.*) ; (*c*) on the pole opposite to the paranuclear body there is also a slightly siderophyl zone, more or less irregular, covering as a cap the same pole and extending to a certain distance in the body. We will name it *polar capsule* (Fig. 2, *c.p.*).

We have said that all the parasites in the red cells show a strong membrane. Very rarely, however, some parasites were seen which did not show this membrane, and were rather constituted by some amount of protoplasm without any trace of periplast : the signification of these forms will be discussed later on.

Let us, for the present, describe the forms provided with this strong membrane. Its constitution is granulo fibrillar, with fibrils and dots

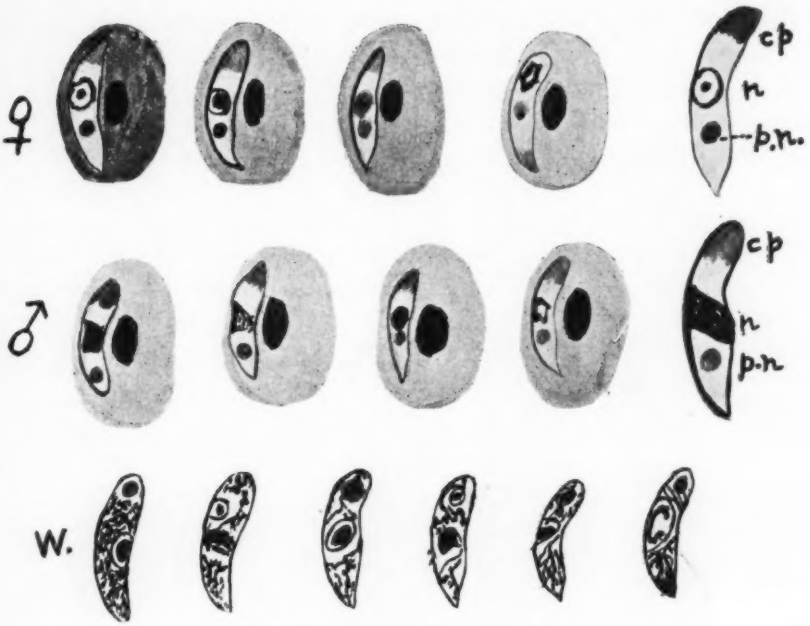

FIG. 2.—Structure of gametocytes.

irregularly interwoven and our Fig. W (Fig. 2) gives an idea of the structure of the wall of this membrane.

The forms provided with this strong membrane have been interpreted by us as *gametocytes* and they can be differentiated into males and females according to the structure of the nucleus : in males the nucleus is compact, whatever be its form, oval or quadrangular. Whatever be the degree of differentiation, one can never distinguish in the endosome any special granule. Entirely different is the constitution of the female nucleus : a definite centriole is seen in its midldle, reduced sometimes to a minute granule, sometimes a little larger. The situation of the nucleus is always central in male gametocytes, often polar in female ones.

Studies of the parasite in the sections of liver and lungs.—The sections were stained by Giemsa, Hæmalun of Mayer, and iron-Hæmatoxylin of Heidenhain. Their study can be divided into two parts.—

I—parasites seen in the red cells of the capillaries.

II—parasites seen in the endothelial cells.

In the red cells.—Moreover, some gametocytes of the same type as that on peripheral blood, a large proportion of parasites are found without the

strong membrane which characterises the former. They were also rarely detected, as we have said above, in the peripheral blood, but their proportion in capillary circulation is very large. Their constitution is

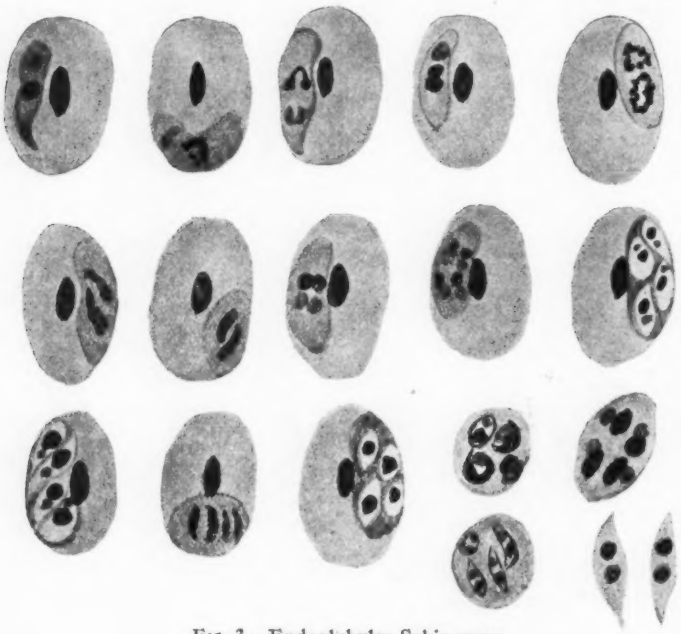

Fig. 3.—Endoglobular Schizogony.

otherwise identical: central nucleus, but with no distinct centriole, paranuclear body and polar capsule.

These forms have been interpreted by us as *schizonts* and they undergo in the interior of the red cell a process of binary division ending in the formation of a cyst containing *four large gregariniform merozoites*. Some figures suggest that the process of the nuclear division is a kind of rudimentary mitosis. The paranuclear body suffers a binary division and it seems that this division is prior to that of the nucleus; but we have not enough evidence for definitely stating this point.

The parasite becomes roundish or oval and shows in the interior of the erythrocyte four merozoites, provided with nucleus and paranuclear body. The situation of the paranuclear body varies to a great extent: often it is situated in the concavity of the nuclear endosome which is curved as comma.

This cyst, which we will name *macrocyst*, becomes free: its whole contents are not spent for the formation of the *gregariniform merozoites* which

finally become also free and invade now the endothelial cells. In these parasites the polar capsule is not always seen distinctly.

In the endothelial cells.—The endothelial cells of the lungs but specially those of the liver are now infected. The *gregariniform merozoite* occupies the protoplasm and generally shows a polar capsule. One cell may be infected by more than one parasite.

The nucleus of the parasite undergoes a process of division ending into the formation of a cyst containing eight *leishmaniform merozoites*. The cyst may become free or rupture in the interior of the endothelial cell (Fig. 4, *a*).

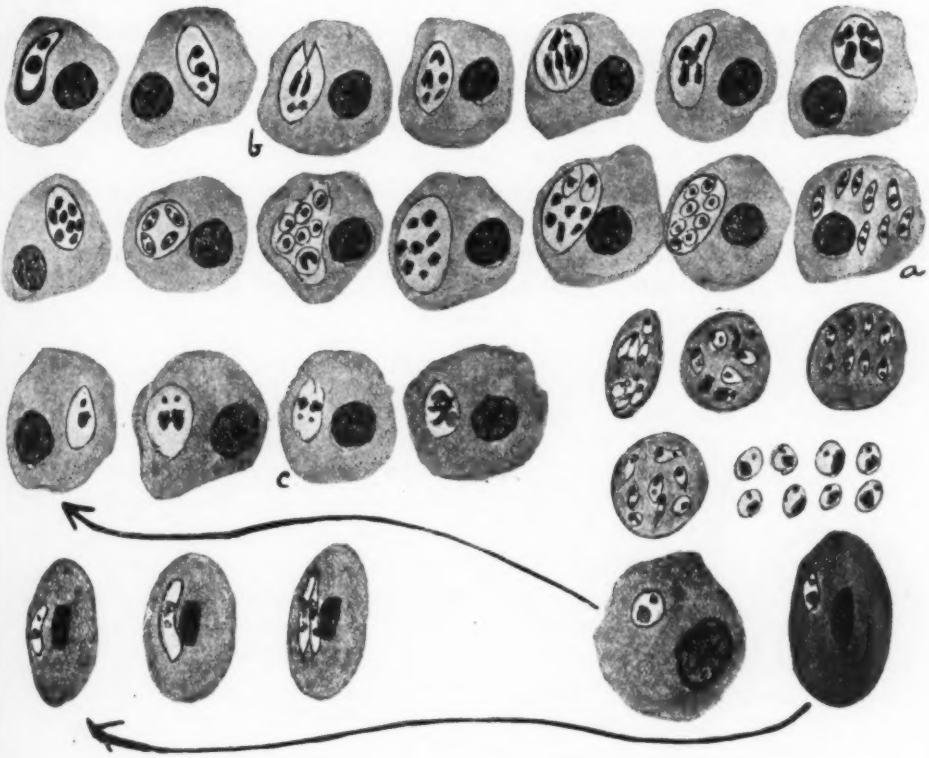

Fig. 4.— Endothelial Schizogony.

The parasites formed in this way are sometimes fusiform, sometimes oval. Will it constitute a signal of primary differentiation of some kind ? We are unable to say. Anyhow their structure is leishmaniform : macronucleus and micronucleus, this one probably representing the paranuclear body,

but not showing a less siderophyl tinctorial reaction as in the schizonts provenient of the endoglobular schizogony. It is quite possible that in such minute forms the paranuclear body is reduced to the siderophyl granule which we often find attached to the paranuclear mass referred to in describing the gametocytes.

What is the ulterior evolution of these leishmaniform merozoites?—They invade both red cells as endothelial cells. In the former they grow up and undergo a process of binary division.

In the endothelial cells, they grow up and become again microcysts.

Is there any differentiation between these leishmaniform bodies which invade both these cells? Indeed in red cells they assume a gregariniform, sausage-like appearance and in endothelial cells a more oval form. But the most accurate examination of the leishmaniform bodies could not show any noticeable difference and in the interior of many microcysts the merozoites assume either an oval or fusiform shape, indifferently.

Another point deserves attention: in all mature microcysts we have counted eight merozoites, which seems to emerge from repeated binary division. Is it possible that this number is sometimes reduced to only two? Fig. 4, *b* and *c* suggest the viability of such hypothesis, but we were unable to find on this point a definite evidence.

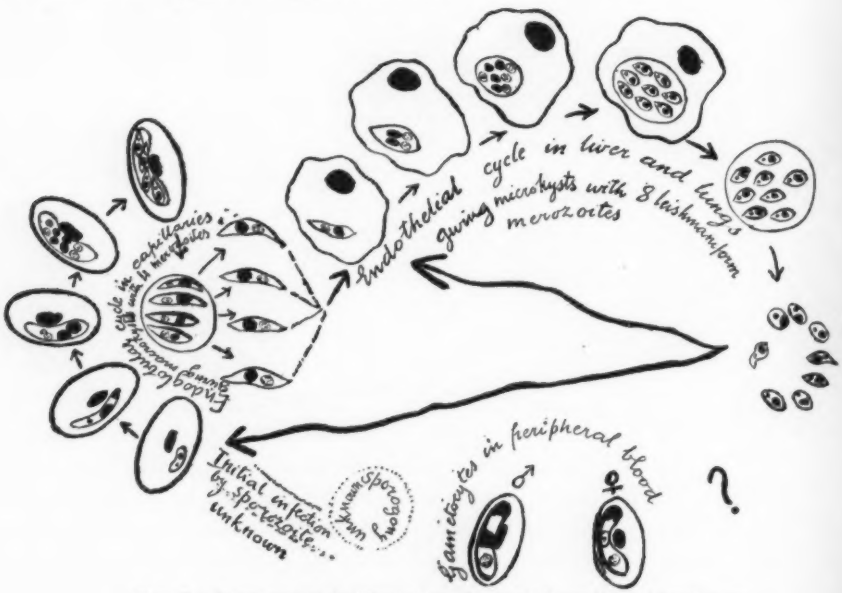
Endothelial cycle in liver and lung's giving microbysts with 8 leishmaniform merozoites
Cycle in capillaries with 8 merozoites
Endoglobular cycle with 8 merozoites
Initial infection by sporozoite unknown
Transmission by sporozoa
Gamétocytes in peripheral blood
♂
♀
?

FIG. 5.—Diagram of the Evolutive Cycle of *Karyolysus jorgei*, n.sp.

Summarising.—The summary of our work on the schizogonic cycle of this parasite is schematised in Fig. 5, and may be resumed as follows :

1. In the capillary circulation occurs the endoglobular cycle : a merozoite invades the cell, grows up and after repeated binary division gives a macrocyst which becomes free and liberates four gregariniform merozoites.

2. These merozoites invade the endothelial cells of the lungs and liver and growing up produce microcysts containing eight leishmaniform merozoites. These microcysts liberate the merozoites or in the tissular interstices or in the protoplasm of the endothelial cell itself. This second schizogonic cycle is called by us the endothelial cycle.

3. The leishmaniform merozoites may invade either the red cells where they undergo further binary division or the endothelial cells where they become again microcysts.

4. In peripheral blood the red cells harbour male and female gametocytes, these ones possessing a definite centriole.

5. It is not known from what forms derive the gametocytes neither whether the leishmaniform merozoites show some differences according to the cells which they invade : red cells or endothelial cells.

6. Sporogony unknown : and hence the sporozoite phase as well as the initial stage of cell invasion through sporozoites unknown.

Classification of the Parasite.

A. Generic classification.—The unpigmented hematozoa inhabiting the blood cells, specially the erythrocytes, have been classified *grosso modo* as *Hæmogregarines.* Discovered by Chaussat in 1850 (*Anguilula minima*= *Lankesterella minima*) in *Rana esculenta* and believed by him to be a stage in the life-cycle of a nematode, they were seen in 1891 by Ray Lankester, who thought they belonged to the life-cycle of a trypansome [*T.* (*undulina*) *rotatorium*]. The name *Hæmogregarina* is first employed by Danilewsky in 1885 for the parasite found in the blood of tortoises and lizards. Neveu-Lemaire raises the name to the rank of a family which he designates as *Hæmogregarinidæ* (1901) (syn. *Hæmosporidea* Labbé, 1894), *Hæmosporea* Minchin (1903). Labbé classifies them according to the length of the parasite in relation to that of the blood cell and on these grounds the three following genera are defined :

(*a*) *Drepanidium*—very small size, the full grown-up vermicule being not more than two-thirds of the length of the erythrocyte.

(*b*) *Karyolysus*—parasite of about the same length as the blood cell or slightly shorter. The name of the genus is derived from the action

of the parasite on the nucleus which is often broken up and karyolysed though not invariably.

(*c*) *Danilewskya*—parasite exceeding the host cell in length and bent upon itself.

The term *Drepanidium*, preoccupied by an Heterokaryota, was altered for *Lankesterella*, the word *Danilewskya* was also altered by Danilewsky in 1897 to *Hæmogregarina* (1885) (*Sensu strictu*).

With these alterations, the following genera are recorded.

Lankesterella Labbé, 1899 (syn. *Drepanidium* Labbé) Sp. typ. *L. minima* (Chaussat, 1850) par. of *Rana esculenta* L.

Karyolysus Labbé, 1814 Sp. typ. *K. lacertarum* (Danilewsky, 1886) par. of *Lacerta muralis* (Laur.).

Hæmogregarina s. *str.*, Danilewsky, 1885 Sp. typ. *H. stepanowi* (Danilewsky, 1889) par. of *Emys orbicularis* L.

Soon Sambon, Miss Robertson and many others describe numerous species which do not agree fully with the three types above proposed. Reichenow describes in 1910 the life-cycle of *Hæmogregarina stepanowi* and every one recognises now that a rational arrangement cannot be proposed until the life-histories of some of these parasites are fully known. Miss Robertson confirms to a certain extent the statements of Reichenow with her researches on *H. nicoriæ* (Cast. and Willey, 1904) par. of *Nicoria trijuga* Schweig. Meanwhile, new parasites are discovered and the names *Hepatozoon*, *Leucocytogregarina*, *Toxoplasma* fill the protozoological literature.

As a provisional groupment the suggestion of Laveran is universally adopted : distinguish only one genus *Hæmogregarina* Danilewsky, 1885, the various species being arranged according to the genera, classes and order of the hosts. And so, in the protozoological books we see now the different species arranged into 4 groups : (i) Hæmogregarinida of Mammals ; (ii) H. of Reptiles ; (iii) H. of Amphibia ; (iv) H. of fishes.

The researches of Nöller (1912–20) show that in the schizogonic cycle of *Lankesterella minima* the rosace-like endoglobular corpuscle giving merozoites, formerly believed by various authors as a stage of *Lankesterella*, belongs to *Dactylosoma ranarum*, another parasite commonly found in the blood of frogs. He describes also the sporogony of *L. minima* as belonging to a typical *Eimeria*. Reichenow, in 1919, individualises among these *Hæmogregarinids* of red cells his genus *Schellackia* (sp. typ. *S. bolivari* Reichenow, 1919 par. of *Acanthodactylus vulgaris* and *Psamodromus hispanicus*), another Hæmogregarine of *Eimeria*-type whose schizogony and sporogony occur in the intestinal wall.

Many of these researches need further confirmation not only for definitively stating the cycles already described, but specially because we are convinced that some of the schemes now commonly inserted in general protozoologic books as typical life-cycles of such and such genus, however, giving a general sketch of the evolution of some of these parasites, constitute rather a specific than a generic character. So, for example, the hæmogregarines of the Indian tortoise which we have described[1] do not show in their schizogonic cycle the stages assigned by Reichenow to *H. stepanowi*. Indeed, if the life-history of all hæmogregarines was known, it would be possible to group them according to a more natural classification ; but, as it is rightly pointed out by Wenyon[2] " in the great majority of cases only the blood forms have been described and as it is not certain whether these are gametocytes, sporozoites or schizonts, it is impossible to determine the genera to which they belong ".

For the present time and considering the research already carried on, the following provisional grouping can be arranged :

I. Macro- and micro-gametocytes of type *Eimeria* : only sporozoites occur in red cells and are transmitted by the invertebrate mechanically and without undergoing further development in the invertebrates body.

Schizogony and sporogony in the endothelial cells of the blood vessels. The rupture of the oocysts gives sporozoites which invade the red cells.

gen. *Lankesterella*.

Schizogony and sporogony in the intestinal wall. The rupture of the oocysts gives sporozoites which invade the red cells.

gen. *Schellackia*.

N.B.—In the protozoology of Doflein[3] both these genera are described as belonging to the family *Eimeridæ*. In the book of Wenyon[2] the order *Coccidiida*, sub-order *Eimeriidea* has among others the family *Lankesterellidæ* Reichenow, 1921 (sporozoites in red blood vessel) and comprises two subfamilies *Schellackiinæ* with a single genus *Schellackia* and *Lankestcrellinæ* with also a single genus *Lankesterella*.

II. Macro- and micro-gametocytes of the *Adelea* type, taken by an invertebrate in whose body occurs the sporogonic cycle ending into the formation of the oocyst which

Schizogony in red blood corpuscles where gametocytes are also formed. In invertebrate host (leech) the oocyst contains free sporozoites.

gen. *Hæmogregarina*.

contains sporozoites. These are conveyed to the vertebrate through the active agency of the invertebrate.

In blood the infected cells are either red or white cells and they may contain either gamétocytes or schizonts.

Schizogony in internal organs, usually in the endothelial cells of the blood vessels. Gametocytes either in red or white cells. Sporogony in the invertebrate (tick, mite, louse) giving oocyst containing a number of sporocysts each of which contains the sporozoites.

gen. *Hepatozoon*.

Schizogony in the endothelial cells. Gametocytes in the red cells. Sporogony in invertebrate (mite) giving oocyst with sporoblasts. These escape from the oocyst as motile vermicules (sporokinetes) and enter the egg in which they become sporocysts within which sporozoites are developed. These enter the intestinal epithelium of the vertebrate through the ingestion of the fæces of the nymph or of the nymph itself, pass to the blood vessels and penetrate the endothelial cells where occurs the schizogony.

gen. *Karyolysus*.

Leaving apart the so-called *Toxoplasma* whose actual position has been discussed by us in another paper[4] we see that in the above grouping a capital importance is given to the syngamic phenomena of these parasites, constituting their sporogonic cycle. Or in those of the *Adelea* type the sporogony occurs invariably in an invertebrate and every one knows how difficult it is to find out the real vector which is often discovered by a mere chance. Does it mean that without these elements it is impossible to classify such parasites ? Of course, not ! In our case, for example, the lizard did not show any ectoparasite at all, and so we must try the classification of our hæmogregarinid only with the elements already collected.

Despite the form and size of the majority of our parasites being in agreement with the old characterisation of *Lankesterella*, our type does not belong to this genus, as no sporogonic stage has been found in the endothelial cells.

Moreover, in the peripheric blood there is every evidence that the bodies inside the red cells show sexual dimorphism and must be classified as gametocytes. In order to render uncontestable the differential diagnosis of our parasite with *Lankesterella*, we will resume the cycle of this genus, supposing that the statements of Nöller are correct and confirmed : according to this author the vermicules of *L. minima* (Chaussat) found in red cells are sporozoites and do not undergo here further development (it is definitively stated now that the rosettes described in the red cells as stages of multiplication of *L. minima* are in reality schizogonic stages of *Dactylosoma ranarum*). The sporozoites sucked by the leech *Hemiclepsis marginata* with the frog's blood enter the epithelial cells of the gut or wander about the tissues without further development. The frog eventually eats the leech and so the sporozoites gain entrance to the intestine of another frog. (*N.B.*—Wenyon states : " in some way not properly understood they gain entrance to another frog ". But in Doflein's *Protozoology* it is clearly written in page 371 : "Die-Rolle des Ueberträgers spielt bei *Lankesterella* der Russelegel *Hemiclepsis marginata*, der beim Blutsaugen die Sporozoitem aufnimmt und der sie dadurch, das er selbst gefressen wird in den Froschdarm gelangen lasst ".) Now begins the schizogony in the endothelial cells of the capillaries first of the intestine, later on in different internal organs such as spleen, liver, lung, bone-marrow. The Sporozoite becomes rounded, commences to grow into a schizont and produces a large number of merozoites, which escape into the blood and infect other endothelial cells. The gamogenesis occurs also in the endothelial cells of the capillaries, specially in pancreas, mesenterium and kidney : merozoites of a special kind develop into macro- and micro-gametocytes. These produce a large number of microgametes and the fertilisation of macrogamete, producing, specially in liver and spleen, oocysts containing numerous (32 or more) sporozoites, which by the rupture of the oocyst escape into the blood and enter red blood corpuscles.

The endoglobular schizogony observed in the internal organs circulation and the characters of sexuality of the endoglobular parasites seen in the peripheral blood, do not allow to classify our parasite in the genus *Lankesterella*.

The same arguments can be applied for differentiating our parasite from the genus *Schellackia*. Created by Reichenow in 1919 for the sp. *Sch. bolivari*, par. of *Acanthodactylus vulgaris* and *Psammodromus hispanicus*, its schizogonic cycle, with the production of ten to sixteen merozoites, takes place in the intestinal epithelium of the mid-gut. In the same place some of these merozoites develop into gamonts. The microgametocytes produce a large number of biflagellate microgametes. The macrogametocytes wander through the epithelial cells and develop in the sub-epithelial connective tissue,

where the fertilisation occurs and the oocyst is formed. This oocyst contains eight sporozoites, devoid of membrane and as it cannot pass to the fæces, as it occurs in a typical intestinal coccidium, ruptures in the connective tissue and the sporozoites, now free, enter the blood stream and penetrate into the blood cells (red cells in *A. vulgaris*, lymphocytes in *P. hispanicus*) where they appear as hæmogregarines. The blood is now sucked up by the lizard mite *Liponyssus saurarum*, whose epithelial cells engulf the infected red cells, digest them, liberate the sporozoites which are now collected in these mite cells, increase a little in size but do not undergo any further development. The mite is eaten by the lizard and the sporozoites now free in the lizard gut infect the epithelial cells and recommence the schizogonic cycle.

Our work could not be complete without the study of the intestine sections of our lizard. They were done to observe the evolution of our parasite in the intestine not only for differentiating it from the genus *Schellackia* but specially because we are convinced that in the schizogonic cycle of this large group of parasites commonly designed as *Hæmogregarina* there are many modalities, often peculiar to every species, which cannot be framed according to the few schemes given by the authors and commonly quoted in general protozoological books.

No evidence of gametes and gamogony of the *Eimeria*-type was found in the intestinal cells of our lizard. Some of the intestinal cells, however, seemed to harbour, rarely, the same micromerozoites and microcysts as in hepatic cells. So our parasite cannot belong to the genus *Schellackia* as defined by Reichenow.

It is very difficult to make an uncontestable differentiation of our parasite from the genus *Hepatozoon*, as this genus has one of its most important characteristics in the constitution of oocysts, which develop in the invertebrate host and possess numerous sporocysts, each of them containing sporozoites. We believe, however, that we are not dealing with a *Hepatozoon* on the following grounds : (*a*) no *Hepatozoon* has yet been described among the Lacertilia. These parasites are harboured specially by mammals, excepting perhaps the *Hepatozoon adiei* studied by Hoare in an Indian unidentified eagle, the *Leucocytogregarina amadinæ* studied by Fantham in the weaver finch *Amadina erythrocephala* and some of the Hæmogregarines described by Aragäo in Brazilian birds, which classified by Nöller as *Toxoplasmids*, are supposed by Wenyon to be of the same nature as the *Hepatozoon adiei* of Hoare, a point on which the senior author of this paper has already expressed his doubts[4]; (*b*) the leucocytes of our lizard were not seen harbouring the parasite as it happens with almost all *Hepatozoon* of mammals, excepting

in *H. gerbilli* Cristophers, par. of *Gerbillus indicus* and in some forms of *marsupialia* which occur in the red cells and whose classification whether pure *Hæmogregarines* or *Hepatozoa* is rather doubtful.

So it is between *Hæmogregarina* sens. strict and *Karyolysus* that we must classify our parasite.

In the genus *Hæmogregarina* s. *str.* no intra-endothelial stage has ever been found. Reichenow and Robertson who have given the schemes of the evolutive cycles of *H. stepanowi* and *H. nicoriæ* describe macro- and micromerozoites occurring only in blood red cells either in the peripheric or internal organs circulation. In the studies of the hæmogregarines of the Indian tortoises we have not seen also any endothelial stage. Therefore, despite one part of the schizogonic cycle occurring in the red cells of internal organs circulation, the existence of an endothelial cycle keeps our parasite away from the genus *Hæmogregarina* type *stepanowi*. It is much more alike to to the genus *Karyolysus*, between which and the latter it constitutes a kind of transitional form.

The genus *Karyolysus* (*Caryolysus*) Labbé owes its name to the fact that the nucleus of the host cell is karyolysed and often fragmented but this action pointed out by Labbé as characteristic of the genus cannot be regarded as of generic value (Wenyon,[2] pag. 1095). In Doflein[3] (pag. 919) it is stated that there is always some action on the nucleus of the host cell, variable in form and degree: lysis, fragmentation, deformation or displacement. Reichenow[5] (pag. 616) says, referring to some karyolysing hæmogregarines: "Wenn auch die Wirkung des Parasites auf die Wirtszelle kein systematisches Merkmal ist, so ist es doch moglich, dass in diesenahnlichen Wirkung eine Verwandschaft der betreffenden Hamogregarinen Zum Ausdruck kommt."

Indeed the lack of karyolitic action of our parasite together with the endoglobular schizogony leading to the formation of macromerozoites makes it a remarkable transitional form between *Hæmogregarina* and *Karyolysus*.

The life-history of the type species, *K. lacertarum* (Danilewsky, 1886), has been fully described by Reichenow. Schizogonic stages in capillaries of intestinal organs and more restrictly in the endothelial cells, where the schizont is surrounded by a membrane. Schizogony by repeated nuclear divisions. In all the stages, moreover the nucleus, there is a *binnenkorper* which stains less deeply and suffers also the division by dumb-bell constriction. Eight to thirty merozoites whose size and details of structure vary considerably in different cysts and which escaping into the blood stream invade other endothelial cells and repeat the schizogony. After schizogony

has been repeated a number of times, the schizonts from which the gametocytes derive, give rise to a large number of merozoites (unterscheidensich von den anderen dadurch, das bei gleicher Zellgrosse *Zalreichere* Merozoitem in ihnen gebildet werden) (Doflein,[3] pag. 916). These merozoites are without binnenkorper and invade the erythrocytes where they are surrounded by a fairly tough membrane, and in such encapsulated stage have the nucleus in polar position (in den eingekapselten Parasiten puckt der Kern an das End Zell. Doflein,[3] *ibid.*). The female gametocyte shows again a binnenkorper located in the pole ; the male one not, but in this too in the intestine of *Liponyssus saurarum* " auch ein kleiner binnenkorper nachweisbar ist " (Doflein,[3] pag. 918). One does not understand really from what source does this binnenkorper in males raise : it seems more logical to admit that during the endoglobular stage it is very minute, rather virtual and such differential structure between male and female gamonts constitute a specific rather than a generic character.

The dimorphism of the merozoites has been observed by many authors and the names of *macro-* and *micro-*merozoites often employed. Labbé himself, who found the cysts specially in the spleen, described in *K. lacertarum* two kinds of schizonts : one with large merozoites (macromerozoites), another with small ones (micromerozoites). It is pointed out by Wenyon that[2] (pag. 1109) " the larger forms are probably the true merozoites which again become schizonts, while the small ones are young gametocytes which enter the red corpuscles ". He may be right but the schizogony which Labbé describes in red cells does not agree with the cycle of Reichenow. Minchin (6 pag. 255) after having stated that " the schizont often becomes surrounded by a membrane, forming a so-called cytocyst ", writes : "In many Hæmosporidia of cold-blooded animals there appears to be a well-marked dimorphism in the schizonts as well as in the merozoites produced by schizogony. Within the cytocyst the schizont may break up into smaller micromerozoites or larger macromerozoites. This occurs in the form *Karyolysus lacertarum* and also in the hæmogregarines infecting various snakes studied by Lutz and named by him *Drepanidium serpentium.* In the latter, the two kinds of merozoites develop into two forms of schizonts termed by Lutz microhæmozoites and macrohæmozoites ".

Laveran and Pettit described similar forms in the spleen and bone-marrow of *Lacerta ocellata* (*Hæmogregarina curvirostris* Billet) and in *Hæmogregarina lacertæ* from *L. viridis* and *L. muralis* of France. Marceau has observed them in his *K. lacertarum* of *L. muralis,* Wenyon in the liver of *Mabuia quinquetæniata* of Sudan [*H.* (? *K.*) *gracilis*]. Wenyon says : "The occurrence of the two forms of schizogony in the liver with the production

of the micro- and macromerozoites is especially interesting as only the micromerozoites are met with in the peripheral blood. It would appear that the macromerozoites live only in the liver cells and there reproduce by schizogony, while the micromerozoites reproduce in the same manner and in the same situation, but also are able to attack the red blood corpuscles.....It is possible that the forms in the blood are undifferentiated gametes, the end products of a special sporogony in the liver and that the larger forms really represent the asexual forms which continue multiplying in the liver by schizogony.[7] The same dualism of cysts has been noted by Short[8] in *Agama tuberculata* and in two Persian lizards.[5]

B. Specific determination.—Trying to identify our species we will first of all sketch an historical review of what is known under the designation *Karyolysus lacertarum* whose meaning has varied according to the authors. A paper of França[11] has been for us of a great value for this purpose, specially because he quotes *ipsis verbis* some words of former authors whose memoirs are not available in libraries in India.

Labbé, studying the blood of *Lacerta muralis*, *L. agilis* and *L. ocellata* of France, classifies all the parasites of these lizards in two genera : (*a*) *Karyolysus lacertarum* par. of all those three species ; (*b*) *Danilewskya lacazæi* pars. of *L. agilis* and *L. muralis* (1894, Labbé, A., *Recherches zoologiques et biologiques sur les parasites endoglobulaires du sang des vertebrés.* Thése de la Fac. de. Sciences de Paris). He identifies *K. lacertarum* with *Hæmogregarina lacertarum* described by Danilewsky and Chalacknikow in *L. agilis* and *L. viridis* of *Karkow* (1886, Danilewsky, B., *Recherches sur la parasitologie du sang III. Les hématozoaires des lézards.* Archives slaves de Biologie). Or such an identification is very doubtful, as Danilewsky described under the name *Hæmogregarina lacertarum* at least two species which he designed under the names *Hæmocytozoon a* and *c*. The simultaneous presence in the blood of those lizards, and the existence of another so-called intermediate form (*form b*) which, designed by Danilewsky as *Drepanidium lacertarum*, is considered by França as a young stage of one of these hæmogregarines, led the Russian author to include both these Hæmocytozoon *a* and *c* under one single species. And from these statements derived the greatest confusion which continued later on.

The work of Labbé increases the confusion as he assembles different Hæmatozoa of 3 species of lizards under one single species, his *Karyolysus lacertarum*, despite having seen that : "les formes adultes sont elles mêmes assez differenciées. Les unes (probablement le parasite *a* de Danilewsky) sont renflées, avec un léger recourbement d'une des deux extremités ; les

autres sont cylindroïdes, se creusent de profondes cavités dans le globule, se colorent fortement.....montrent un noyau souvent peu visible ; d'autres intermediaires sont allongées, cylindriques, fusiformes et montrent des granulations terminales disposées de diverses façons." But Labbé concludes: "toutes ces formes se résument en un même type : une petite Hæmogrégarine aux formes plus lourdes et moins effilées que le *Drepanidium* atteignant, mais ne dépassant pas la longueur du globule sanguin, c'est-à-dire ayant de 11 à 14 microns ".

In 1901 Marceau, following the same criterion adopted by his predecessors, describes a capsulated hæmogregarine in *L. muralis*, identifies it to *K. lacertarum* Labbé, but expresses some doubt on this point as he says: " tout cela, bien entendu, en admettant avec Labbé qu'il n'y ait qu'une seule espèce de parasite ".[12] The parasite of Marceau takes the form of cylindroid masses, slightly bent in arch, rarely with a pointed and curved extremity, 15/5-6 microns. Nucleus rarely visible, ovoid, spheric or parallelipipedic. Young free forms in liver, some of 8/2 (microsporozoites), others of 12/3-4 (macrosporozoites). Red cell hypertrophied, nucleus fragmented.

In 1907 Finkelstein describes her *K. berestenewi* from the caucasic *L. muralis* : cucumber-like form, with a very long pointed tail describing often some complicated turns in the interior of the red cell which is hypertrophied as well as its nucleus. The tailed part of the parasite is 4 to 5 times longer than the larger part.

In 1908, Laveran and Pettit[13, 14] describe the Hæmogregarinids of *L. viridis* and *L. muralis* of France, identify their parasites with those found by Danilewsky and Chalachnikow in 1886 in *L. viridis* and *L. agilis* from Kharkow and following the same criterion propose for all the forms they met with the name *Hæmogregarina lacertæ*. They describe three forms which they consider evolutive stages of the same parasite : small forms ; middle ones, 10/2, cylindric, roundish poles, but one of them slightly pointed and bent, nucleus sub-central nearer to the roundish pole, protoplasm light blue at Giemsa, red cell hypertrophied, nucleus displaced and often fragmented; large forms with aspect of " vermicules qui répliés dans les hématies mésurent 14-15/2-3. Le parasite a d'ordinaire une extremité plus éffilee que l'autre, il est replié tantôt à une extrémité seulement, et il s'agit alors de l'extrémité effilée, tantôt aux deux extermités, autour du noyau de l'hématie. Le protoplasme prend une teinte violacée qui s'explique par la présence de nombreuses granulations chromatophiles ". Red cells hypertrophied, their outline granular or finely denticulated and the protoplasm containing chromatophile granulations. Nucleus hypertrophied, fragmented and often thrown away from the cell.[13]

França arrives to the conclusion that excepting for the species of Marceau and Finkelstein which have enough characteristics for retaining their validity, the authors have started from a wrong idea, *viz.*, that a single species of Hæmoprotozoon is harboured by different species of hosts and so the so-called *Karyolysus lacertarum* (= *Hæmogregarina lacertæ*) is a designation including not one, but many zoological species.

França having studied 80 infected specimens of *L. muralis* var. *fusca* from different regions of Portugal has found and well characterised various species of *Hæmogregarines* in their blood. He gives the following synopsis of the parasites of *L. muralis* :

I. Red cell hypertrophied.

A. Without capsule.

Cytoplasm generally violet ; small chromatic dots in the poles ; one of these poles large, whilst the other is pointed and curved. Multiplication cysts in liver. 19,5-22,5/4,5-5,5 microns. *H. nobrei* França.

B. With capsule.

(1) Capsule surrounding the parasite. Multiplication cysts in liver. 15/5-6 microns. *H. sp.* Marceau.

(2) Cucumber-like, the pointed extremity very long and describing often complicated turns. 28/5 microns not counting the tail. Multiplication ? *H. berestnewi* Finkelstein.

(3) Blue cytoplasm ; large red semispheric cap covering each pole. Multiplication cysts in organs, specially liver. 10,5-12/3,5-4,5 microns. *H. bicapsulata* França.

(4) Sexual dimorphism shown by violet and blue cytoplasm of endoglobular forms. Capsule rose, covering the convex part of the parasite. Multiplication forms preceeded by conjugation. 9,5-10,5/3-3,5 microns. *H. marceaui*, França.

II. Red cell not hypertrophied.

Cytoplasm pale blue, chromatic granules. 5,2-6/3,5 microns. Multiplication ? *H. nana* França.

The so-called conjugation forms described by França in *H. marceaui* are a kind of syzigy, where two capsulated hæmogregarines are united together and fused up[11] in his figs. 12, 13, 14, Pl. III. From this conjugation—which does not seem to us evidently proved—should derive the macro- and microcysts : the ones 22-24/18-19,5 containing 13-23 sporozoites, violet

protoplasm; the others, 16-18,5/13,5-15 containing 6-9 sporozoites, blue protoplasm.

Concerning the action of these parasites on red cells we have the following: *H. bicapsulata*—red cell decolorised, full of red granules, nucleus karyolysed and fragmented; *H. marceaui*—red cell decolorised, hypertrophiated, nucleus hypertrophied and paler than in normal cells; *H. nana*—no alteration in the red cell; *H. nobrei*—red cell strongly hypertrophied, nucleus displaced, seldom fragmented in two parts.

Woodcock expressed the view that all the forms occurring in the lizards *Lacerta muralis* and *L. ocellata* belonged to the same species, but this statement does not agree with the observations of other protozoologists. Laveran and Pettit[15] say: Labbé described *K. lacertarum* (= *Hæmogr. lacertæ*) in *Lacerta ocellata, agilis, muralis* and var. *tangetana* from Algiers; Nicolle described *H. biretorta* (1904) in *L. ocellata* var. *pater* from Tunis; França described in *L. ocellata* from Portugal *H. schaudinni* (1908) and *H. nicollei, H. minuta* (1909). He gives the characters of the Hæmogregarina examined by him in *L. ocellata* var. *pater* from Tunis: (*a*) endoglobular vermicules, 11-14/1-1,5 microns, one pole a little narrower, nucleus central; (*b*) same forms a little larger and with rare chromophyl granulations in the protoplasm; (*c*) large forms generally capsulated, alveolar protoplasm with many chromophyl granules, one extremity bent. Red cell hypertrophied, are in large forms decolorised, their nucleus elongated and often fragmented. Cysts in *bone-marrow in one specimen* and in *spleen* in *another*. They measure 18-28/12-22 and contain 8-16 macromerozoites or 48 or more micromerozites and are found always in capillaries and never " sur les frottis (foie, rate poumon) ni dans les coupes des éléments pigmentés de grande dimension, renfermant des *H. liberes* ou des kystes, *contrairement* à ce que nous *avone noté pour L. lacertæ*". And identifying this parasite with *H. curvirostris* Billet which they definitely differentiate from *H. lacertæ*, they express some doubts on the validity of the ssp. *biretorta, nicollei, schaudinni* var. *africana* which they are rather inclined to homologize with *H. curvirostris*, accepting however as valid the ssp. *H. schaudinni* and *H. minuta* of *L. ocellata* from Portugal.

In another paper[16] Laveran and Pettit, describing the parasite of *Psammodromus algirus*, identify it with *H. psammodromi* Soulié despite the measurements somewhat smaller and write: " *H. pallida* França qui ne mésure que 6 à 7, 5 de long et qui n'exerce aucune action nuisible sur les hématies ne peut être confondue avec *H. psammodromi*; quant à *H. lusitanica* son existence, en tant qu'espèce distincte de *H. psammodromi, ne nous* parait pas démontrée."

The subject of infestation of the same vertebrate by different sspp. of the so-called hæmogregarina is no more doubtful in present days. Reichenow writes in Doflein's *Protozoology* (3 p. 919): "Allein in *Lacerta muralis* kommt eine gross Anzahl von *Karyolysus-Arten* vor, die auch in Mischinfektion autreten Konnen und von denen allen die sporogonie in der gleichen Milbenart erfolgt. Die Entwicklung zeigt insofern Verschiedenheiten, als bei einem Teil der Arten, wie bei *K. lacertæ* in der Darmwand, bei einem anderen Teil in der Leibeshohle der Milbe, vorwiegend in den Zellen der Hypodermis erfolgt. In Darme entwickeln sich Z. B. *K. bicapsulatus* (França) und *K. zuluetai* G. Reichenow". Reichenow[17] describes in *L. muralis* from Madrid no less than eight species of hæmogregarines which are different from the species studied by him in this lizard in Italy.

Mme. Physalyx[18] writes: "on sait d'autre part qu'une même espèce animale peut être infestée par plusieurs hémogregarines différentes ainsi qu'il arrive en particulier chez les crotales et le *Bufo agua*". In *Crotalus terrificus* she finds "deux hemogrégarines différentes et différant chacune aussi de celle qui a été décrite par M. A. Laveran chez une autre espéce du mème genre; *Crotalus confluentus*".[19] In *Bufo agua* Latr. (= *Bufo marinus* Lin.) there are four sspp. of hæmogregarines.[20] From this long discussion one can see that:

(*a*) many hæmogregarines may infect one single species of cold-blooded vertebrates;

(*b*) often the hæmogregarines found in one country differ from those found in another country;

(*c*) often the schizogonic cycles of the same hæmogregarine occcur in different organs according to the infected lizard. In *H. butantensis* this cycle has been found in the sections of heart.[21]

(*d*) different hæmogregarines of the same morphological type have often their schizogony in different organs of the same mite.

Taking into consideration all these remarks, the *characterisation of the species* must obey primarily to a detailed morphological structure of the endoglobular elements, their action on the red cells and secondarily to the structure of their cysts and the organs in which they occur. Important, first of all to note, the species of the host as, for the present at least, there are no arguments of experimental infection and cross immunity to let us believe that to one single species of hemogregarine belong a lot of parasites of this kind as it was supposed in former days.

* * *

On this ground we must state:

1. No hæmogregarine has been previously described in the blood of *Calotes versicolor*.

2. Our parasite does not alter the red cell, the only blood element which is parasitised by this *Karyolysus*.

In this respect we find in the literature which we were able to consult:

(*a*) *H. pallida* par. of *Psammodromus algirus*[22] oval, pyriform or banana-shaped, inequal poles, 6-7,5/3-4 microns. Protoplasm pale blue or rose with chromophyl granules. Nucleus band-like, perpendicular to the long axis. Young forms oval or elliptic in mononuclears, in hepatic pigmented cells of the liver, measuring 4,5/2,2-3. Red cells not altered.

(*b*) *H. nana* par. of *L. muralis*. Elliptic or pyriform, 5,2-6/3-3,5. Protoplasm colourless or slightly blue, showing in one pole, often in both, small chromophyl granules. Nucleus band-like. Schizogony unknown.

(*c*) *H. chamæleonis* par. of *Chamæleon vulgaris* [23]: falciform, banana-shaped, 3-4-7/1-1,5, seldom parasitising also white cells, nucleus central, rose or almost black, protoplasm blue; often one pole larger lodging the nucleus, which is then sub-central. No blepharoplast, no other organelle in the protoplasm which is vacuolated. Red cell not altered.

(*d*) *H. sp.* par. of *Tarentola mauritanica* from Cyrenaica.[24] Banana-shaped, 12-14/4-5. Protoplasm blue, nucleus roundish or quadrangular, central or slightly sub-central; plenty free forms, almost tailed. Red cell not altered.

N.B.—The same author found in *Tarentola mauritanica* from Yemen another parasite quite different, pyriform, roundish or oval, with one vacuole in the centre, 10-12/5-6 or 6-8 for roundish forms; nucleus attached to one border of the parasite. Red cells hypertrophied; its nucleus displaced and hyperstained.

(*e*) *H. sp* par. of *Agama stellis* from Rodi. Red cells not altered in young forms but elongated in adult forms. Nucleus not altered.[24]

N.B.—The parasite of *Agama stellis* Tripolitana, studied as that from Rodi by Tuddia is completely different; banana-shaped, with larger poles, 12·13/3-3,5 microns; (4-5 microns on poles). Protoplasm light blue. Central nucleus, light coloured. Red cell slightly hypertrophied and discoloured, its nucleus elongated.[24]

(*f*) *H. missoni* par. of *Tupinambis teguixim*, 8-9/2; 5·3. Considered as young form of *H. tupinambis* Laveran and Salimbeni. Studied by Carini, 1909. Red cells not altered. No other information found.[25]

(*g*) *H. minuta* par. of *Lacerta ocellata*. Studied by França. Red cell not altered. Other informations not found.

(*h*) *H. leptodactyli*. Studied by Lésage, 1908, the present informations have been taken from Cunha and Muniz.[26]

Two kinds of cysts surrounded by a membrane: (*a*) with 2-8 elongated merozoites, slightly bent, sausage-like, 16-20/6-10, with reticulate plasma, nuclear chromatine irregularly disposed and karyosom not distinct; (*b*) with 16-32 or more small merozoites, 2,5-3 microns broad, dense plasma without any noticeable structure, large band-like nucleus with chromatin around the nuclear membrane or forming a net schizogony of macromerozoites in the interior of the organ cells (intestine, liver and lung, specially in both these organs) and they enter new cells and repeat the schizogony (agamogony). After some time of similar multiplications, they give rise to the schizogony of micromerozoites and these, liberated in the blood current, enter the red cells, constituting the endoglobular gametocytes, which do not show, however, any sexual differentiation. The schizogony does not occur in the endothelium of blood vessels. Nucleus of red cells not altered.

(*i*) Our parasite: Red cells not altered and harbouring in peripheral circulation, male and female gametocytes provided with a strong capsular periplast and measuring 5-7-11/1,5-2,5 microns. Rarely in peripheral circulation, but frequently in internal circulation the red cells harbour schizonts which do not show any periplast and whose nucleus suffers a binary division giving 4 macromerozoites or gregariniform merozoites measuring 8-11/2,5-3 microns. The macrocyst measures 13-15/8-10. The small leishmaniform merozoites, provenient of endothelial microcysts measure: roundish forms 1,5-3 microns; fusiform merozoites 3-5/1-1,5.

Many of the hæmogregarines above quoted possess an intracellular multiplication in their evolutive cycle. *Hæmogregarine procteri* Shortt par. of *Phyllodactylus elisæ*[9] shows schizogony with micromerozoites in the red cells of lungs, spleen and liver, contained in cysts of 6/5 and a 2nd schizogony with macromerozoites in the endothelial cells of lungs (cysts 15/12, macromerozoites 6,5/1,8). Our parasite has many interesting peculiarities which enable us to classify it as a new sp. The existence of a *paranuclear body* so often figured in *K. lacertarum* and always present in the various evolutive stages of our parasite (even in leishmaniform merozoites under the form of a strongly siderophyl micronucleus), the presence of a polar capsule in gametocytes, the evident sexuality of gametocytes, the endoglobular and endothelial cycles of schizogony, the peculiar shape and structure of the micromerozoites have not been described in any hæmogregarinid hitherto recorded.

We will therefore consider our parasite as a sp. n. which will be named *Karyolysus jorgei* in homage to Prof. A. Ricardo Jorge, President of the XII International Congress of Zoology, held at Lisbon, in 1935.

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Coloured Plate No. VI will appear in Vol. V, No. 5, Section B.

AN ANALYSIS OF THE SHAPE AND STRUCTURE OF GOLGI BODIES IN THE EGGS OF INVERTEBRATES WITH A NOTE ON THE PROBABLE MODES OF ORIGIN OF THE GOLGI NETWORK.

By M. K. Subramaniam, M.A., D.Sc.,
and
R. Gopala Aiyar, M.A., M.Sc.
(*From the University Zoological Research Laboratory, Madras.*)

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Introduction.

Our purpose in writing this paper is not merely to record our observations on the shape and structure of the Golgi apparatus in the eggs of Invertebrates but also to suggest some probable modes of formation of a network from these various shapes. We make this attempt because we consider that the cytologists working on the phenomena of oogenesis have ignored many of the suggestions of students in other branches of the study regarding the shape of the Golgi apparatus, notably those of Bowen ('20, '22 *a*, '26 *d* & '26 *e*).

This lack of a clear understanding of the shape and structure of the Golgi apparatus in oogenesis is reflected even in the reviews on the subject (MacBride and Hewer, '31) and hence in this paper we have made an attempt to put the available facts from our work into a connected whole, taking into consideration the already established facts in spermatogenesis and secretory phenomena.

Literature.

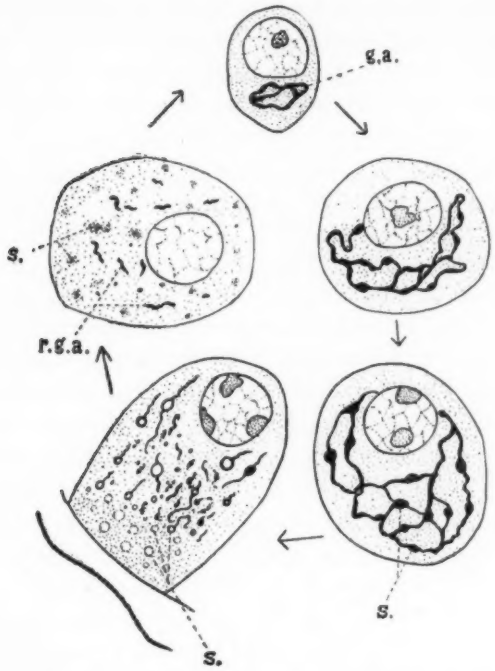
The historical review of the whole subject up to 1926 treating with the works of Holmgren ('03, '08 & '10), Golgi ('98), Cajal ('14), Duesberg ('12 & '14), Bensley ('10), Voinov ('25), Morelle ('24 & '25) and others has already been made by Bowen ('26 *e*) and hence we intend to refer to these papers only when they have some bearing on the problems with which we are concerned in this paper.

During the course of our investigations on the phenomena of yolk formation in eggs we came across Golgi bodies having different shapes. In *Clibanarius* (Subramaniam, '35 *a*) and *Stomopneustes* (Subramaniam and Gopala Aiyar, '36 *b*) we observed during the growth of the egg, the gradual transformation of a Golgi granule into a batonette. The granules which had no

differentiation into chromophilic and chromophobic regions enlarged into vesicles showing the two regions clearly. These by rupture gave rise to batonettes which reminded one of similar bodies described in invertebrate animals by Hirschler ('13, '14, '16 & '18), Gatenby ('17 *a*, *b*, *c*, '19 *a*, *b*, *c* & '22), Bowen ('20, '22 *a*), Beams and Goldsmith ('30) and Harvey ('31 *a* & *b*). Moreover Gatenby ('17 *a*) and Hirschler ('18) described rings and rods lying side by side. Even though Hirschler described ring and half-ring shaped Golgi elements in the oogenesis of *Ciona intestinalis* ('16) and Bowen ('26 *e*) from his review concluded that this cellular constituent may assume almost any shape and distribution, a reticulum being only one expression of its protean appearance, the polymorphic condition of the Golgi apparatus in eggs has been much disputed even as late as 1931. Nath in 1925 described in the earliest oocytes of Scorpion " a few clearly-defined curved rods lying on one side of the nucleus". But in the case of *Pheretima* (Nath, '30), *Periplaneta* (Nath and Mohan, '29), *Otostigmus* (Nath and Hussain, '29), *Culex* (Nath, '29) and *Dysdercus* (Bhandari and Nath, '30) he describes vesicles and especially in *Pheretima* he attempts to interpret the various other shapes that he observes as caused by improper impregnation, optical sections, etc. In his latest paper (Nath, '33) he mentions that the Golgi apparatus is polymorphic but we are not able to decide whether that applies during oogenesis also. Harvey on the other hand describes "Argentophil vesicles and irregular masses" in *Ciona* (Harvey, '27) but in his latest paper ('31 *c*) he remarks while criticizing Nath's work that " there can be no connecting link between these vesicles and the netlike Golgi apparatus characteristic of vertebrate cells, which is the fundamental absolute to which all questions of the form of Golgi apparatus must ultimately be referred" (p. 451). He continues " there is one form of Golgi apparatus only in invertebrate cells, that of an ensemble of dictyosomes" (p. 452). How different is this conception from that of Bowen ('26 *d* & *e*) who considers the Golgi apparatus as a specialized cytoplasmic substance which may be moulded into any shape demanded by particular cellular conditions !! That hollow spheres have an existence may be seen from Hirschler's work ('18) whose main consideration in postulating his " Apparatinhalt " was that in the case of hollow spheres the central contents could not possibily be the same as the external cytoplasm. Students of oogenesis seem to consider oogenesis as a special phenomenon and do not refer to papers on spermatogenesis and secretory phenomena. As pointed out in another paper (Subramaniam, '35 *b*) we believe rather that the aim of all work on the structure or function of the Golgi apparatus should be to correlate, if possible, results obtained in one field with those recorded in others. The Golgi apparatus of secretory cells is in no way different from that found

in the various other tissues of the body, because it is the Golgi bodies in the eggs which give rise by division and distribution to the Golgi apparatus in all the cells of the body. Hence we are faced with the question, how the Golgi bodies having the same origin can be attributed different shapes and function in different cells. Either the diversity of behaviour, shape and structure should be accepted or a *de novo* origin of the Golgi apparatus in different types of cells should be proved. The latter possibility being negligible, the only recourse will be to accept the former one. The more we learn about the Golgi apparatus even in eggs, the more we are convinced that the variations are only the variations of a single basic procedure. Harvey's remark that the Golgi apparatus can have only the dictyosome shape disregards the work of Hirschler and Gatenby. Gatenby even in 1917 describes batonettes giving rise to vesicles. He says " At the time the mitochondrial bodies begin to run together, the acroblasts which are about three or four in number, gradually become vesicular. The process is quite easily followed out and can often be seen occurring in several acroblasts in one spermatid" (p. 439). A look at his figures reproduced below (Text-Figs. 21, 22, 23 & 24 *a*, *b*) will show that both the types, dictyosome and vesicle, occur side by side. Figures illustrating Gatenby's work on *Saccocirrus* ('22) especially his figures 1, 4, 19, 20, 21 & 32 will amply convince any one that there could be no hard and fast rule regarding the shape of the Golgi apparatus in oocytes, spermatocytes and spermatogonia. Similarly in secretory cells also, the diversity of shape could be gathered from the work of Ludford ('25 *a*, *b*, '27 & '31 ; Ludford and Cramer, '27), and Bowen ('26 *a*, *b*, *c*). Harvey fails to consider that even in the case of the boomerang shaped scales sections in different planes may often lead to the production of rings in some particular planes (compare the shapes in Text-Fig. 3 $g.b_2$, $g.r_2$ which is after Bowen, '20). Moreover during secretory activity it has been noticed that the apparatus during hypertrophy assumes a variety of shapes. The network at an advanced stage of secretion has been known to break up into rings, rods and granules (Ludford and Cramer, '27). Below is reproduced the pictographic summary of the secretory cycle in the Islets of Langerhans after Ludford and Cramer (Text-Fig. 1).

As this paper also deals with the structure of the Golgi apparatus we give below a review of the work dealing with the structure as conceived by different cytologists. Even at the outset it may be mentioned that except Hirschler('18) and Bowen ('26 *d* & *e*) few seem to have considered the difference in structure between the network-like Golgi apparatus and the dictyosome. The dictyosomes according to Gatenby and Bowen have a duplex structure consisting of chromophilic and chromophobic parts. But in the network-



TEXT-FIG. 1.—Pictographic summary of the secretory cycle in the Islets of Langerhans. (Fig. 23, p. 23, Ludford and Cramer, 1927.)

like Golgi apparatus of vertebrate somatic cells the chromophobic part seems to be lacking. As even in mammalian germ cells (Gatenby and Woodger, '21; Gatenby and Beams, '35) an idiosome has been demonstrated one naturally inquires how the chromophobic part comes to be absent in the network-like Golgi apparatus. Unless a clearer understanding of the structure of the Golgi apparatus is available a minute analysis of the part played by the apparatus in secretion is not possible. We have always been faced with the question whether the secretion of the acrosome is comparable to the secretion of mucus, serous and lipoid granules in secretory cells. It will be seen from papers on spermatogenesis (Bowen, '26 *d*) that the acrosome always arises near and in relation to the chromophobic part. No case has been recorded in which the chromophilic part is next to the acrosomal vesicle or granule and the chromophobic part farther away and having no relation with the vesicle or granule. In spite of the differences of opinion among students of oogenesis all are agreed (Nath, '29, '30 & '33 ; Harvey, '27, '31 *a*, *b* & *c* ; Subramaniam, '34, '35*a*, '37 ; Subramaniam and Gopala Aiyar, '35, '36*a* & '36*b*) that fat, fatty

yolk and yolk when they are formed as products of the activity of the Golgi apparatus always arise in relation with the chromophobic part of the dictyosomes or vesicles. Such being the case, the fact has to be admitted that the chromophobic part plays as essential a role as the chromophilic part in cells where the apparatus has a duplex structure.

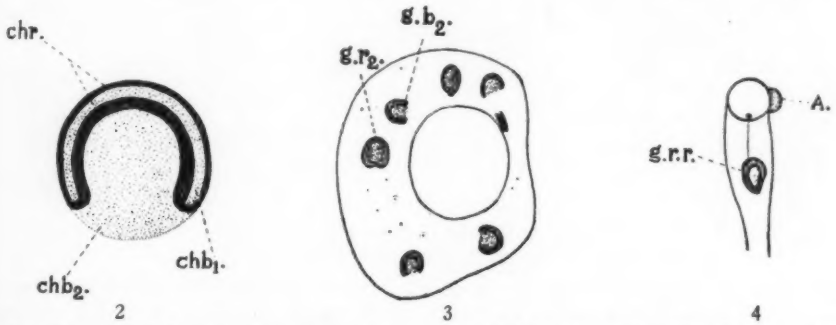
If this is so, how is the secretion of the acrosome comparable to the secretion of mucus, serous and lipoid granules in secretory cells? Bowen in his papers definitely mentions that in the network he could never find the chromophobic component. Hence in order to compare secretory granule formation the first problem to be solved is the process how the chromophobic part disappears. We have come across a phenomenon (Subramaniam and Gopala Aiyar, '36 *c* & *d*) by which a vesicle by infolding gives rise to a dictyosome with double rims in which the two parts by closer apposition give rise to Golgi batonettes in which no chromophobic part is visible.

Harvey's ('31*b* & *c*) main objection as pointed out before is the method by which the vesicle gives rise to a network. His statement ('31*c*) that "The netlike Golgi apparatus of most vertebrate cells is foreshadowed in the vortex-like complex forms assumed in young eggs, of which *Antedon* provides an excellent example and in the somatic cells of many adult forms (*vide* Hirschler, 1914, 1918)" (p. 452), clearly demonstrates that he has not considered the problem of the structure of the Golgi apparatus at all. Hirschler ('18) though he had considered the duplex structure (his Apparathulle and Apparatinhalt) has not interpreted the loss of the chromophobic part in the networks. In addition to all these Nassonov ('24), Ludford ('25) and Bowen ('26*a*, *b*, *c*) record double-rimmed batonettes, hollow tubular networks and vacuolated networks. Are all these artifacts or are they only the variations of a single basic structure? If they are real they may, in a way, give a clue to the solution of the duplex structure of the Golgi bodies.

Even networks found in secretory cells are different from those found in cells of tissue cultures and tumour cells. In tissue culture cells Ludford ('27) records both a network and a collection of granules. Ludford, while discussing the structure of the Golgi apparatus, says that though he finds a chromophobic component in relation with the network, yet as the chromophil threads become drawn out, he has been unable to make out any differentiation into the two above-mentioned regions. Similar experience is also recorded by him in connection with the changes in the form of the apparatus during hypertrophy in the cells of the fibroblasts of tar tumours. His own interpretation ('27) is that "It is possible in both these cases that the idiosomic substance is either spread in a thin film over a chromophil core, or it may be

that the two are combined in such a way that the two substances are not distinguishable optically" (p. 418). His conclusions regarding the disappearance of the chromophobic portion as presented in his paper on the tar tumours are slightly different. He is of opinion that the chromophobic part may persist "as a fine layer surrounding the reticulum of the apparatus or else it may form the basis of the reticulum over which the material of the apparatus is spread in a similar manner to the insulating material covering an electric wire" (p. 569, 1925*b*). Whatever may be the process there should be intermediate stages between the one with the duplex structure and that with the chromophobic part either inside or outside. Ludford and for the matter of that no worker offers any evidence which may suggest the possibility of there being intermediate stages between the discrete bodies and the network.

Now we shall mention those papers in which double-rimmed batonettes, tubular networks, and vesiculated networks are mentioned. In the spermatogonia of *Euschistus euschistoides* (Bowen, '20) the Golgi bodies presented a characteristic appearance (Text-Figs. 2, 3 & 4). Viewed on edge the individual



2 3 4

Text-Fig. 2.—Schematic representation of a single Golgi body. (Text-Fig. 1 A, p. 329, Bowen, 1920.)

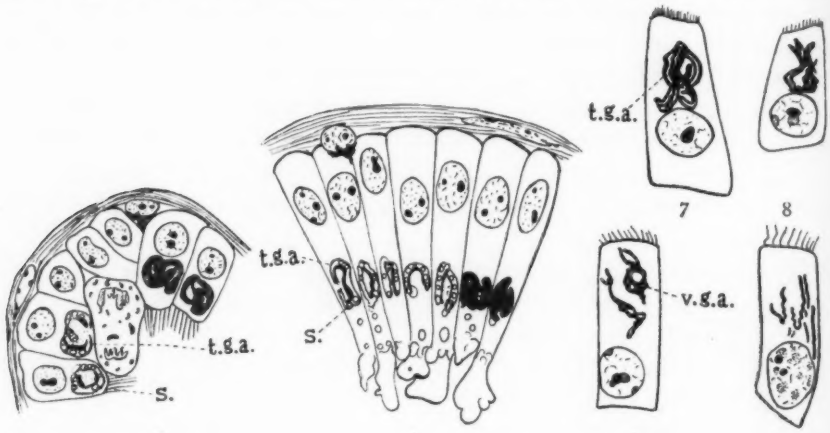
Text-Fig. 3.—*Euschistus euschistoides*. Spermatogonium late growth period. Note the double rim in all the bodies and also ring and rod shapes lying side by side. (Pl. 1, Fig. 7, Bowen, 1920.)

Text-Fig. 4.—*Brochymena quadripustulata* spermatids showing Golgi remnant. (Pl. II, Fig. 31, Bowen, 1920.)

Golgi bodies gave the impression of a pair of rods lying side by side (Text-Fig. 2 *chr.*), and separated by a clear space (*chb*$_1$.). This slit was observed by Bowen from the very early stages upto the condensation and even the Golgi remnant (Text-Fig. 4 *g.r.r.*) in his figures shows this identical structure. It is possible that such a feature may be of wide occurrence for it is more frequently figured but rarely referred to in several published accounts of the Golgi

apparatus. Bowen is certain that the division of the Golgi bodies is always at right angles to the slit and the latter therefore however well adapted it may be for purposes of division never serves that end. Even after the fusion of the Golgi bodies to form a single acroblast, the structure has a double rim as could be made out from Bowen's figure 31 (Text-Fig. 4 *g.r.r.*).

Nassonov ('24) and Ludford ('25*a*) while working on the secretory phenomena in the epidydymis came across Golgi networks composed of hollow tubes. Figures representing the hollow nature as drawn by them are given below. Text-Figs. 5 and 6 are from Nassonov's paper and Text-Figs. 7, 8, 9 and 10

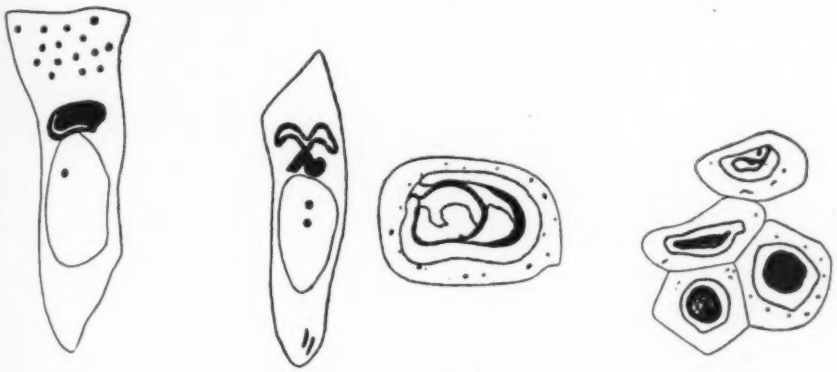


Text-Figs. 5 & 6.—Epidydymis of the mouse showing tubular Golgi networks with secretory droplets inside such strands. (Nassonov, 1924.)

Text-Figs. 7, 8, 9 & 10.—Stages in the secretory cycle in the epidydymis. Note the tubular nature of the network in Figs. 7 and 8. The vacuolated condition is seen in Fig. 9. (Figs. 15, 16, 17 & 21, p. 363, Ludford, 1925*a*.)

are from Ludford's. The liquid form of secretion (*s.*) is mentioned by both as originating inside these hollow tubes (*t.g.a.*). Bowen re-examined the phenomena in the epidydymis of the cat and the rabbit and observes that he also found examples similar to Nassonov's figures in great numbers in the various parts of the male reproductive tract of both the cat and the rabbit. In addition, he found another closely related appearance in which the blackened Golgi contours were bordered externally by a light zone similar to the one which, in the other cases, appeared on the inner border of a blackened contour. Some of his figures illustrating these appearances are shown in Text-Figs. 11, 12, 13, 14, 15, 16, 17 & 18. He has observed these identical appearances even in longitudinal sections (Text-Figs. 15 & 16). As no relation existed

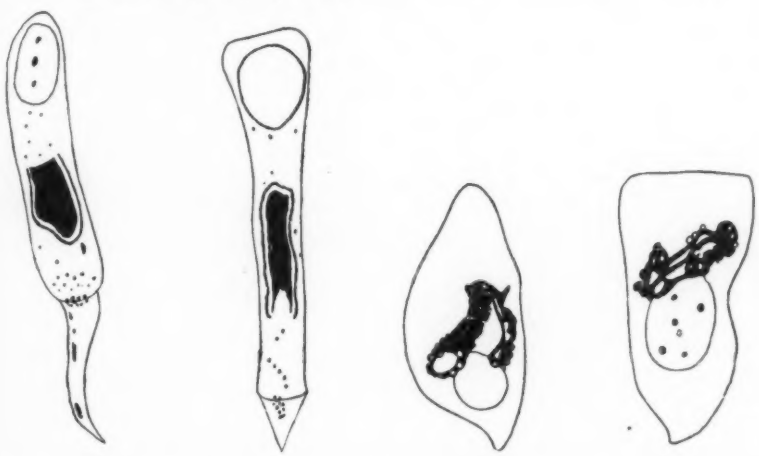
between secretory granules and these spaces Bowen feels that these appearances may be artifacts caused partially by the fixative due to the massive development of the Golgi apparatus in these cells. Even after suggesting that such appearances result from shrinkage, he considers it possible that such a



11 12 13 14

TEXT-FIGS. 11 & 12.—Golgi strands having a tubular appearance. Vas deferens of the Rabbit. (Pl. 28, Figs. 87 and 86, Bowen, 1926*c*.)

TEXT-FIGS. 13 & 14.—Golgi contours bordered by a light zone. Both are cross-sections. (Pl. 26, Figs. 33 and 48, Bowen, 1926*c*.)



15 16 17 18

TEXT-FIGS. 15 & 16.—Golgi apparatus having a tubular appearance. Longitudinal section of a cell from the proximal portion of the epidydymis of the Cat. (Pl. 26, Figs. 39 and 40, Bowen, 1926*c*.)

TEXT-FIGS. 17 & 18.—Vesicular Golgi networks in some cells in the vas deferens of the Rabbit. (Pl. 28, Figs. 83 and 85, Bowen, 1926*c*.)

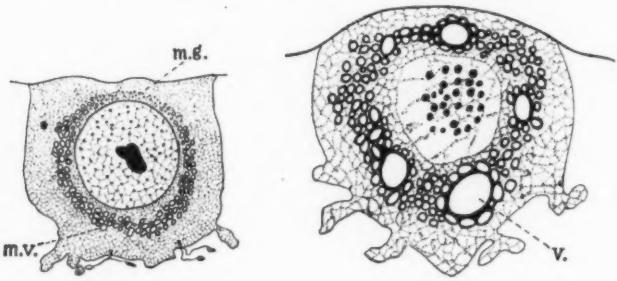
tendency to react to osmic acid will eventually throw some light on the intimate structural details of the Golgi apparatus.

It is a wonder that after having described double-rimmed batonettes in *Euschistus* he considers the double outlines of the Golgi bodies in the salivary gland cells of *Limax* as pure artifacts. In cross-sections of secretory cells of the vas deferens of the rabbit, Bowen records some interesting details. The network in such sections lacked uniformity of diameter and consisted of blackened materials connected together by delicate cords. When the impregnation was light these networks presented a vacuolated appearance. In Text-Figs. 17 and 18, we have reproduced some of the appearances seen by Bowen. Such appearances, he remarks, give one a distinct impression that the swollen masses of the Golgi network are formed by a cluster of small vacuoles. He continues " Such vesicular developments occur in other gland cells, but I know of none where these appearances are so prominent and of such frequent occurrence. Much interest attaches to the interpretation which is to be given to these vesicles. Are they artifacts caused by some swelling action of the fixatives or are they actually the beginnings of secretory granules? The latter conception would immediately explain the appearances and demonstrate in a remarkable way the relation between the Golgi material and the secretory products which, it is suggested, are differentiated under the action of the Golgi apparatus. Definite evidence of the reality of these vesiculated Golgi masses would obviously provide the final demonstration of the views which I have elsewhere suggested (Bowen, '24). Unfortunately in the present instance there are reasons for admitting the possibility that the appearance is an artifact, and while it is hard to believe that such an explanation can be complete, nevertheless in a critical case of this kind it seems best for the present to be very cautious " (pp. 414–15, '26 *c*). That these vesiculated masses have a real existence may be gathered from Ludford's paper. Even though he does not refer to such vesiculated masses in the text, yet figures such masses in his figs. 17 and 19 (Text-Fig. 9 *v.g.a.*). Bowen does not seem to have considered the possibility that the chromophobic part may become differentiated inside some of the strands of the network. We say chromophobic part, because it is the part which has been shown to be almost in intimate relation with the acrosomal vesicle as also with forming fat and yolk droplets in eggs. Ludford's later work on the Islets of Langerhans ('27) as also in vital staining of acinar cells of the pancreas ('31) clearly demonstrates the possibility that in some cases at least the secretory or segregation products may arise inside the strands. Figures in his pictographic summary of the behaviour of the Golgi apparatus during secretion in the Islets of Langerhans cells make our suggestion clear even though Ludford does not refer to

any such differentiation into chromophilic and chromophobic parts in this particular case. Three workers working on the epidydymis and vas deferens and seeing an identical structure constitute a sure proof of the reality of its appearance especially as one of them (Ludford) figures them without making any mention of the occurrence in the text of the paper. Bowen concludes by expressing the belief that the idiosomic substance is a derivative of the Golgi material and that while it may become very clearly individualized as in male germ cells, it may in others be latent or may be so intimately related to the lipoidal constituent as to be lost in the impregnation of the latter. It will be of considerable comparative interest that the differentiation not to be made out in somatic cells is also experienced in a few cases in the Lepidoptera and Grasshoppers. Such a conception according to Bowen while accounting for observed facts also permits the possibility that in secretory cells " the idiosomic or chromophobic material may be more or less differentiated within the Golgi area, but invisible with the technique employed. I am inclined to think, however, that such a development does not exist " (p. 438, '26 *d*). We have not been able to decide whether Bowen's statement " differentiated within the Golgi area " means differentiated inside the strands or between the strands. The latter seems to be Bowen's opinion for, if differentiation is inside the strands of the network the invisibility will be due to optical difficulties and not due to technique. Whatever may be the interpretation Bowen does not seem to have believed in the possibility of the chromophobic part forming a core to the strands of the network. Nassonov's view that the Golgi material forms a surface of separation between the granule and the surrounding plasma throws some light on the structure of the Golgi apparatus. Even though he does not seem to have paid much attention to the structure of the apparatus his suggestion mentioned above is in accord with his observations. Nassonov's suggestion of the origin of secretory droplets is more comparable to that of acrosome formation than Bowen's own modification of Nassonov's view. Many of the instances mentioned above suggest that the chromophobic part may form a core around which the chromophilic part may lie just like an insulating material. But in the majority of the cases the core may be absent. We have to consider now how the chromophobic part comes to lie inside and also whether there are any recorded instances of such behaviour.

In the numerous papers dealing with the behaviour of the Golgi bodies and mitochondria scanty attention has been paid to structure. Even though pointed references are absent there are in studies on the behaviour of the mitochondria instances where threads originate from vesicles. As mitochondria are also polymorphic occurring in the form of grains, vesicles and threads

we believe that references to recorded instances of change of shape from vesicle to thread form may not be out of place. Gatenby's and Bowen's papers on Lepidopteran and Insect spermatogenesis give a clue as to how thread formation may take place. We have to point out here that as in the case of Golgi granules, mitochondrial granules also when they enlarge have been noticed to differentiate into chromophobic and chromophilic regions. Gatenby's figure (Text-Fig. 19, *m.g.*, *m.v.*) of the process given below will make it clear that even as early as 1917 the process has been described. The above-mentioned paper is especially interesting in that Gatenby figures and describes some peculiar behaviour of the mitochondria. According to him near the end of growth stage the mitochondria have a tendency to run together to form vesicles as shown in our Text-Fig. 20 at V. He seems to believe



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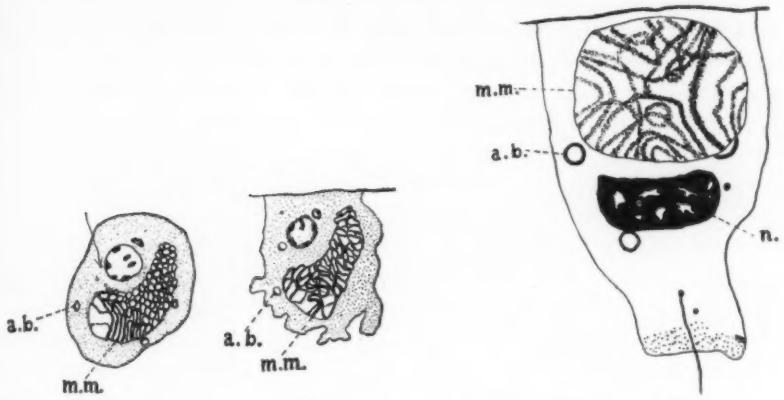
Text-Fig. 19.—Spermatocyte of *Smerinthus populi* showing mitochondrial granules and vesicles. (Pl. 23, Fig. 7, Gatenby, 1917*a*.)

Text-Fig. 20.—Equatorial view of the first maturation division of *Smerinthus populi* showing precocious running together of mtiochondria. (Pl. 23, Fig. 11, Gatenby, 1917*a*.)

that in such cases the running together may be caused by the close contact and subsequent fusion of the outer rim of the mitochondrial bodies and the flowing together of the chromophobic fluid core of the mitochondria taking part in the formation of such large vesicles. While the macromitosome is formed the following behaviour is described. After the second maturation division while the nucleus is becoming reorganized the mitochondrial bodies show a tendency to collect in a horn-shaped mass near the nucleus. This preliminary massing being over there is a tendency for the chromophilic rims to flow together and give rise to a spireme. The most significant fact to be observed here is that while the outer layer forms the spireme, the inner chromophobic areas which have coalesced together form the substance in which the spireme lies. The whole process could be made out in Text-Figs. 21, 22 and 23. Having traced the behaviour of the mitochondria so far



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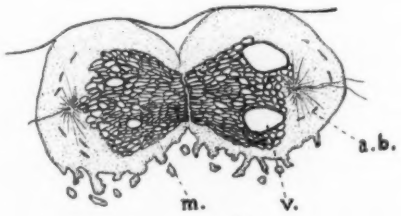
TEXT-FIGS. 21 & 22.—The mitochondria running together to form the macromitosome. Acroblasts are becoming spherical. (Pl. 25, Figs. 35 and 36, Gatenby, 1917*a*.)

TEXT-FIG. 23.—Spermatid showing macromitosome, nucleus and acroblasts. (Pl. 23, Fig. 15, Gatenby, 1917*a*.)

we shall now go back to the behaviour of the same cell organ during spermatogonial division. During division the mitochondria are affected by what is taking place in the cell. Some of them become elongated in the longitudinal axis of the spindle and others run together with their neighbours and also become elongated in the same direction. Text-Figs. 24 and 25 which are taken from Gatenby's paper illustrate this behaviour very clearly especially in the anaphase and telophase. In Text-Fig. 24 is shown the mitochondria forming funnel-like masses with their narrow ends applied to each other representing the region where the cells constrict. Gatenby suggests that such elongation might be due to mere mechanical reasons, such as the pressing of individual mitochondria one against the other.



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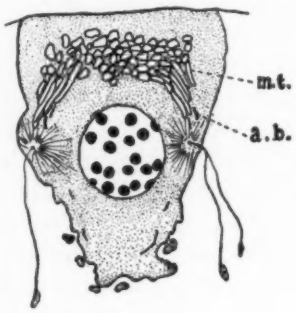
TEXT-FIG. 24.—Spermatocyte in first maturation division showing vacuoles formed by coalescence of a number of mitochondria. It will be seen that the individual mitochondria in the funnel-like mass are elongated. (Pl. 25, Fig. 32, Gatenby, 1917*a*.)

Gatenby records some other peculiar processes also. In addition to the formation of large vesicles by fusion of a number of smaller mitochondrial vesicles filament formation also occurs. In places where the mitochondria are densest they run together to form filaments and cords as shown in Text-Figs. 25 and 26 *m.t.* His explanation seems to be " that such peculiarities are due to the effect of the fixative on the matter around which the true mitochondrial substance is applied ; thus the filamentous condition, of which Pl. 25, fig. 31 is an example, is probably due to the rupture of the outer layer by the ' brutality ' of the fixative and the consequent running together of the rims of the mitochondria. This last process is one which the mitochondrial bodies always have a tendency to undergo. It may, then, be stated that the running together, though a natural process in later stages, is artificially hastened by the action of the fixative " (p. 424, '17 *a*). We shall now consider whether the elongation and running together is a precocious exhibition of a similar power possessed by them in later stages of spermatogenesis or whether they are artifacts. Gatenby records coalescence and formation of large vacuoles as a natural process. He further records that these mitochondrial bodies finally fuse together to give rise to the macromitosomal spireme. If these two are natural processes, Gatenby's evidence for the running together of the mitochondria during division stages as artifacts is not sufficient. As Gatenby's later work has demonstrated variation even among the spermatogonia of the same generation we feel that there are reasons to consider the thread formation and elongation as caused by a precocious exhibition of a latent power. That mutual pressure and other mechanical causes do not explain such lengthening of the vesicles may be gathered from his fig. 48 (Text-Fig. 25) which is in the prophases of the first maturation division. Brutality of fixation does not also explain why the mitochondria in the centre should have run together to form rods while the mitochondria in the other regions instead of being distorted in various ways retain their vesicular shape. Figs. 49 and 31 in his paper are not comparable since in Fig. 49 all the mitochondria have been distorted. What happens to the chromophobic parts of the vesicles when they become filamentous is not clear from his explanation. Do these filaments have the chromophobic part outside or does the chromophobic part come to lie inside the filaments and thus become obliterated during the process of elongation ?

A second method of thread formation has been observed by Meves in the honeybee ('07) and Gatenby in *Tenebrio* ('19). In the latter Gatenby believes that " the mitochondria either exist as spheres containing a chromophobe zone inside or else as elongated tubes containing internally a chromophobe substance. These tubes are formed by fusion of granules and when

viewed under monocular vision look like apposed solid rods which are really hollow tubes (I use hollow in the sense that their centre is chromophobe, though it contains a fluid) " (p. 251, '19 *a*).



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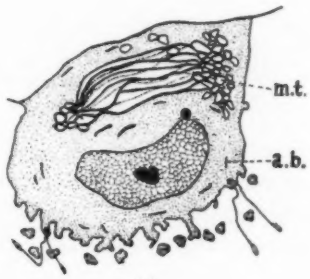

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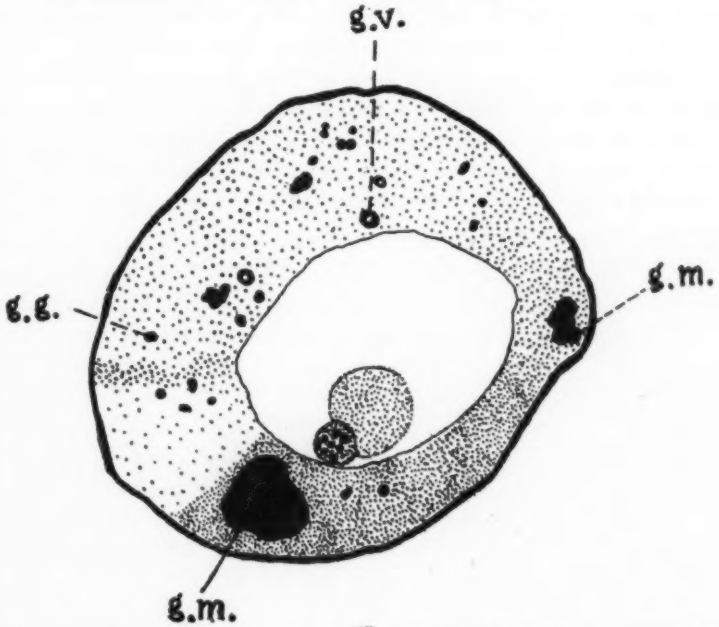
TEXT-FIG. 25.—Prophase of first maturation division in *S. populi* showing elongated mitochondria. (Pl. 25, Fig. 48, Gatenby, 1917*a*.)

TEXT-FIG. 26.—Spermatocyte in which the mitochondrial vesicles have given rise to thread-like mitochondria. (Pl. 25, Fig. 31, Gatenby, 1917*a*.)



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TEXT-FIG. 27.—An early growing oocyte of *Meretrix* showing Golgi masses, Golgi granules and Golgi vesicles. Mann Kopsch × 2,000 (Original).

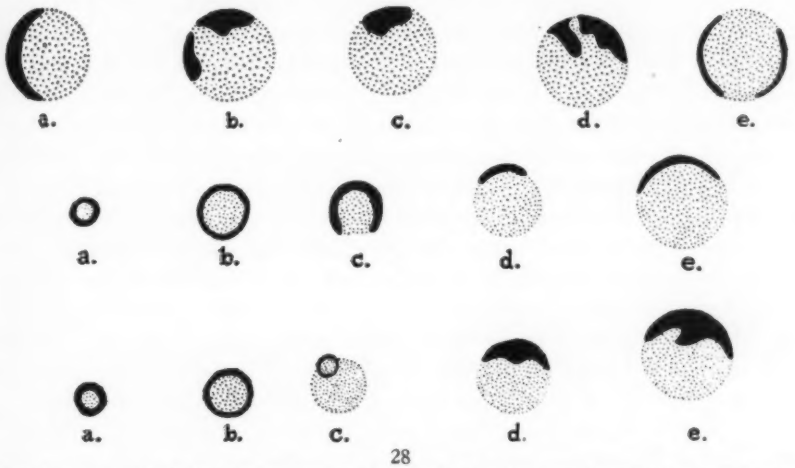
Observations.

During the later stages of the study of oogenesis in various animals, we paid particular attention to the structure of the Golgi bodies. In addition we have examined many of our old preparations. In *Clibanarius* (Subramaniam, '35 *a*) and in *Meretrix* (Subramaniam, '37) the earliest oocytes contained a Golgi mass in which no differentiation into chromophilic and chromophobic regions could be observed (Text-Fig. 27 *g.m.*). In *Salmacis* (Subramaniam, '34), *Meretrix* and *Clibanarius* the initial mass breaks up into a number of granules. This granular condition in which the individual bodies have no duplex structure is retained in *Salmacis* throughout oogenesis. In *Meretrix*, *Stomopneustes* (Subramaniam and Gopala Aiyar, '36 *b*) and *Clibanarius* the granular components of the Golgi apparatus give rise to a number of vesicles. The processes leading up to the formation of vesicles is interesting in *Meretrix*. Innumerable variations in behaviour remind one of the variations observed in the cells of Islets of Langerhans (Ludford and Cramer, '27). The initial Golgi mass divides into two one of which generally migrates to the other side of the nucleus. One of the two masses so formed may divide again. These masses may immediately become resolved first into a number of granules and later into a number of vesicles or one of them before the others may give rise to vesicles with a chromophobic interior (Text-Fig. 27 *g.g.*, *g.v.*). The initial mass may break up into a number of vesicles before division. Such a collection of vesicles may divide into two and both may begin to scatter their individual elements at the same time or one group may remain inactive for a longer time and may even divide into two. In a few cases, the vesicles never divided but began to scatter from the place of their occurrence.

In *Stomopneustes* (Subramaniam and Gopala Aiyar, '36 *b*), *Clibanarius* and *Dasychone* (Subramaniam and Gopala Aiyar, '36 *a*) rod-like dictyosomes occur. But the three differ in that in *Dasychone* the scale-like dictyosomes alone are present throughout oogenesis whereas in both *Stomopneustes* and *Clibanarius* dictyosomes arise by rupture of the vesicles. Plate VII, Fig. 1, is from a Nassonov preparation of *Clibanarius* ovary. At *g.g.* are the Golgi grains without any differentiation into chromophilic and chromophobic regions. At $g.v_a$. and $g.v_b$. are vesicles formed by the enlargement of the grains in which the two regions could be seen. At $g.v_c$ is shown the rupture of the vesicle and the resultant batonette could be seen at $g.b_1$. A look at the Plate will show how the chromophobic area enclosed by the chromophilic region in the vesicle comes into relation with the cytoplasm.

In *Stomopneustes* during the secretion of albuminous yolk the Golgi bodies present a variety of behaviour. Their behaviour may roughly be classified

into two different types based on the structure of the bodies when found attached to the mitochondrial clumps. (1) After slight enlargement of the Golgi vesicles rupture on one side takes place resulting in the formation of semi-lunar shaped elements as in the case of *Clibanarius*. (2) In some cases the Golgi vesicle attached to a mitochondrial clump becomes irregularly plastered on the mitochondrial clump without rupture. Whereas in the first case the chromophobic part comes into relation with the mitochondrial clump, in the second instance the chromophobic part is not visible at all. The different shapes assumed by the Golgi after the different modes of attachment is diagrammatically represented in Text-Fig. 28.



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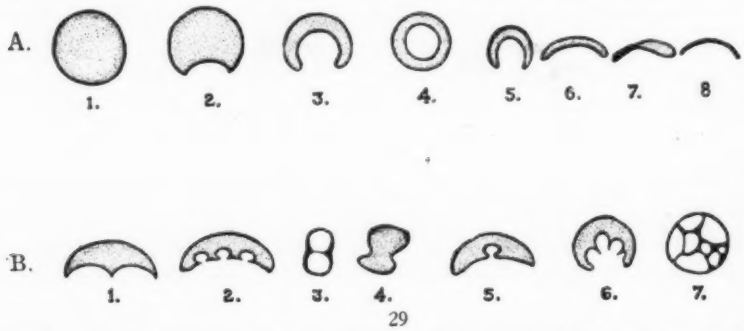
TEXT-FIG. 28.—1. Various shapes assumed by the Golgi when found attached to mitochondrial clumps. 2. A diagrammatic representation of the formation of a Golgi batonette by rupture of a vesicle and its attachment to a mitochondrial clump. The chromophobic part becomes reduced to invisibility during the process. (Figs. 2 and 3, p. 579, Subramaniam and Gopala Aiyar, 1936*b*.)

One of us very recently (Aiyar, R. G., '35) showed that *Lycastis indica*, a common polychæte worm, occurring in the brackish waters in and around Madras, is a hermaphrodite. Its eggs are highly plastic and we thought that a study of the phenomena of oogenesis in *Lycastis* would be of interest (Subramaniam and Gopala Aiyar, '36 *d*). We are recording below some peculiar behaviour and structure of Golgi bodies which have not been recorded previously in eggs. Incidentally, we may mention here that the Golgi bodies could be seen even in Champy fixed material. The Golgi bodies in the growing oocytes could be seen as vesicles. These vesicles during the growth of the egg become converted into double-rimmed batonettes. In Plate VII, Fig. 2,

the stages leading up from the vesicle to the double-rimmed batonette could be seen. At *g.v.* is shown a typical vesicle showing chromophilic and chromophobic regions. This vesicle by a process of tucking in gives rise to a gastrula-like structure which later gives rise to a double-rimmed batonette (Plate VII, Fig. 2, Plate VIII, Figs. 3 & 4). The whole series could be seen in Plate VII, Fig. 2. At $g.v_1$. is shown the beginning of the process of folding. This becomes deeper and deeper ($g.v_2$. & $g.v_3$.) and the resulting double-rimmed batonette could be seen at $g.b_2$. Thus the batonette comes to have the chromophobic part between the two chromophilic rims. In Plate IX, Fig. 5 at $g.r_2$. and in Text-Fig. 29 A at 4 could be made out sections of the same bodies at right angles to the plane in which they lie in Plate VIII, Figs. 3 and 4. In Plate IX, Fig. 5, the mitochondria are visible as scattered granules in the cytoplasm. Finally in the fully grown oocytes, the majority of the batonettes do not seem to have any chromophobic component at all. Such absence of the chromophobic part has been observed by Nath in Scorpions ('25) and Bowen and others in a few Lepidoptera and Grasshoppers. Plate VIII, Fig. 4, shows how the chromophobic part is completely absorbed. At $g.b_{2x}$. is shown a double-rimmed batonette in which the two rims have united at one of the ends. This process goes on till no chromophobic part is discernible. A pictographic summary of the process is given below (Text-Fig. 29 A).



29

TEXT-FIG. 29.—A diagrammatic representation of the formation of a double-rimmed batonette as well as a batonette in which the chromophobic part is absent from a Golgi vesicle. At 4 is shown a cross-section of stage 3. (Slightly modified after Fig. 13, Subramaniam and Gopala Aiyar, 1936*d*.)
B. The different methods of multiple folding. At 3 is shown a cross-section of a slightly later stage of infolding than those shown in 1 and 4. At 7 is shown one of the shapes that result after complex multiple folding. (Original.)

Multiple folding of the walls of the vesicles has also been observed (Plate VII, Fig. 2 and Plate VIII, Fig. 3). In Plate VII, Fig. 2, is shown ($m.f_1$.) an instance of double folding. In Text-Fig. 29 is shown some of the

methods of multiple folding observed by us. Similar bodies could be seen in the Plates. Perusal of Plate VII, Fig. 2, Plate VIII, Fig. 3 and Plate X, Fig. 6 and Text-Fig. 29 B, 7, reminds one of the vacuolated networks figured by Bowen, Nassonov and Ludford. It will be seen that in the figure showing the extremely vacuolated condition (Plate VIII, Fig. 3, $m.f_2$., $m.f_3$.) the chromophobic part is reduced to invisibility by the fusion of the walls of the folds lying near.

Discussion.

Even though the Golgi apparatus was discovered some 40 years ago, little is known about its structure. During recent years Parat's papers ('26, '28; Parat and Painleve, '24 *a*, *b*, *c* & '25) diverted the attention of cytologists to a study of the vacuome. Enough work has been done (Gatenby, '29, '32 & '33) to show that the Golgi apparatus is a permanent cell inclusion quite different from the vacuome. Further, we have reasons to believe (Subramaniam, '37) that neutral red vacuoles in eggs which we have examined represent enzymes secreted by the Golgi apparatus.

Passing now to a consideration of the shape of the Golgi bodies we feel that the photographs will convince anyone that the apparatus in eggs may have any shape ranging from that of a granule to a dictyosome. In this we are only bringing the diversity of shape of the Golgi bodies observed by us in eggs into line with facts recorded by a host of workers in cells from other tissues. In many eggs as in *Salmacis* and *Dasychone* a particular shape persists throughout the major part of the growth period of the oocyte. *Dasychone* may only be one of the many animals in which the shape of the Golgi bodies is fixed. But that fact alone does not entitle one to define the shape of the Golgi apparatus in all types of eggs. Gatenby's statement in the case of the oocytes of *Helix* will, we believe, prove that even in 1917 there has been observed variations even in cells lying in a single section. " I have already mentioned that great variation was found in the appearance of the male cells. This, I think, applies even more strongly to the case of female cells....." (p. 588, '17 *c*). That this variation is not restricted to the mitochondria alone may be gathered from his observation "I can, at present, think of at least several sorts of spermatogonia ; by this I mean that it is quite possible to find a large number of cells which are in the spermatogonial generation of male cells and which differ markedly either in their nucleus, their nebenkern or their mitochondria" (p. 571, '17 *c*).

The process of formation of a typical batonette from a granule is analagous to the behaviour of the mitochondria during the formation of a macromitosomal spireme. The only difference between the rupture of the mitochondria and the rupture of the Golgi vesicle seems to be that in the case of the mitochondria

the process is only an intermediate stage, where soon after rupture all the chromophilic portions fuse together end to end. In the case of the Golgi bodies of *Clibanarius* the process seems to stop with rupture. Later, each batonette comes into relation with mitochondrial or Golgi clumps and secretes albuminous yolk.

Golgi bodies with duplex structure and Golgi networks with a conspicuous absence of a duplex structure are comparable to vesicular mitochondria with chromophilic and chromophobic regions and filamentous mitochondria in which the chromophobic part is absent. Yet, a clearer understanding of the origin of the filamentous mitochondria as also of the Golgi network is not yet available. That even in the case of the filamentous mitochondria which have no duplex structure a necessity for some conception that they should have originated from structures with duplex structure may be gathered from Bowen's work ('22 *b*) where the filamentous mitochondria when they form the nebenkern have the power of differentiating a chromophobic portion. In his example Bowen ('22*b*) finds thread-like mitochondria in which he has not been able to find a differentiation into the two components. Yet, after the mitochondrial threads fuse together the chromophobic part puts in its appearance as a number of vacuoles in the periphery of the mitochondrial mass. In *Murgantia histrionica* Bowen finds in the early spermatocyte period mitochondrial granules possessing a duplex structure. He deduces from the above observation that as the vesicles produce the long thread-like mitochondria typical of *Euschistus*, both the parts should be present and that the threads should have an inner core of chromophobic material as in the honey-bee (Meves, '07) and *Tenebri* (Gatenby, '19). Thus even in observed cases of mitochondrial behaviour there are two types of thread formation. No previous worker seems to have given any clear account of the process which leads to the formation of a thread from a granule. In another part of the paper while we were discussing the behaviour of the mitochondria in Lepidoptera, we pointed out that some of the shapes of the mitochondria in Lepidoptera may not after all be due to the action of the fixatives but may be a precocious exhibition of a latent power. We suggest that the elongated tubes shown in Text-Figs. 25 and 26 may after all be stages leading up to the formation of the thread-like mitochondria where the chromophobic inner core is not visible.

Similar to observations in mitochondria there are also recorded instances of double-rimmed batonettes and hollow tubular networks. But Bowen is not prepared to extend his suggestion of the supposed presence of an inner core to the mitochondrial threads, to the strands composing the Golgi network. Even observations regarding the presence and position of the chromophobic

areas are highly contradictory. Bowen in his figures of the double-rimmed batonettes in *Euschistus euschistoides* shows two chromophobic areas: (1) between the two rims (Text-Fig. 2, chb_1.) and (2) in the cup-like area between the two arms of the U of the batonette (chb_2.). The lamellar view of the structure of the Golgi apparatus suggested by Hirschler ('18) and applied by Bowen to the elucidation of the structure of the nebenkern and the Golgi network may explain the figures of Bowen. Such a conception is not considered at all by Bowen in *Euschistus* but that the Golgi bodies have three dimensions may be made out from the different shapes of the batonettes in his figures. A careful examination of Bowen's figures especially his figs. 8, 9 and 10 also demonstrate that the real chromophobic part may only exist between the two chromophil rims and that the apparent chromophobic part between the arms of the U may be the lamellar structure in surface view.

Ludford who studied the net-like Golgi apparatus records the presence of a chromophobic component in relation with the network in tissue culture cells and tar tumours. Ludford's views have been presented elsewhere but the processes in tissue cultures and tar tumours seem to be entirely different. In the fibroblasts of tar tumours the Golgi apparatus which exists in the form of rods having a duplex structure when they anastomose and give rise to the complicated network lose their chromophobic component (p. 569, '25). In the tissue culture cells, on the contrary, the network which has in relation with it an idiosome loses the above part when it draws out into threads. The processes are directly contradictory and both have been observed in tissues which show wide departures from the normal. Bowen in his wide search for the chromophobic part could not come across any network in which he could find an unmistakable idiosomic component. We have discussed in the resume of literature some of Bowen's observations in relation to similar work on similar tissues. There seems to be a distinct possibility that some networks at least differentiate an area during some part of secretory activity which is comparable to the chromophobic component of the discrete dictyosome.

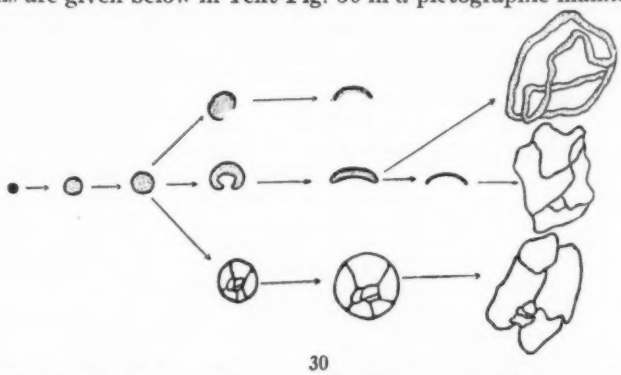
The above considerations lead to the question of the nature and presence of the chromophobic part. Bowen ('26*e*) suggested two possibilities: (1) That the idiosome may arise as a differentiation product of the Golgi apparatus and (2) that the Golgi apparatus is constantly differentiated into two components which are only morphologically distinguishable with the present technique in the germ cells. Though Bowen found it difficult to decide between these two possibilities our results make it difficult to accept both the suggestions. The behaviour of the Golgi grains in the formation of a batonette in *Clibanarius* and the behaviour of the vesicle in *Lycastis* during the formation of a batonette having no duplex structure suggest that the

idiosome is masked in the grain and the network and hence could not be resolved under the powers of the microscope. But when a granule enlarges into a vesicle both the parts become apparent. There also seems to be a relation between the chromophilic and chromophobic regions in the various shapes of the apparatus. Thus the chromophobic part which appears when the grain enlarges into a vesicle may later be reduced to such an extent as to be absolutely invisible as in the case of the dictyosome in *Lycastis*. It will be seen that the above concept is directly opposed to that of Morelle ('22, '24, '25 & '26) who suggested that the space enclosed by the Golgi apparatus as a whole is the Golgi substance, the peripheral layer alone of which is blackened by osmic acid to give the characteristic appearances. And we also find no reason to concur with Beams and Goldsmith ('30) that the chromophobic part is a result of the immediate expression of the metabolic activity of the Golgi body, nor with Voinov's view ('25) that the whole complex is to be viewed as a single thing—the idiosome being without any significance. The above suggestion also differs from Bowen's conception of the structure of the Golgi apparatus. The conclusion arrived at by Bowen is that "the Golgi apparatus is a substance which may be scattered through the cell in the form of discrete bodies, often plate-like in form, or the substance may be concentrated leading to a network with strands sometimes in all probability like mere threads, but at other times certainly spreading out to form extensive lamellar surfaces. In other words, the important thing is that the Golgi apparatus is a substance, the exact modelling of which in the cell is purely a matter of secondary interest". Though agreeing with the lamellar structure of the batonettes and networks, we would like to add to Bowen's description that the idiosome masked in the granule and present in the vesicle is secondarily reduced in the network. In other words, networks, like granules, are occasionally able to differentiate a chromophobic region or tubular networks with a chromophobic core to the strands may be present in some cells.

Having cleared the ground thus far we shall proceed to a consideration of the probable nature of origin of the network. We believe that the papers of Bowen ('26*a*, *b*, *c*, *d* & *e*), Ludford ('25*a*, '25*b*, '27 & '31 ; Ludford and Cramer, '27), Nassonov ('23 & '24), Brambell ('25) and others sufficiently demonstrate that during the final stages of secretion, the typical network becomes broken up into discrete bodies and that a new apparatus is re-organized from these elements (see also Text-Fig. 1). Hence the possibility of a similar origin by union of the discrete dictyosomes becomes at once apparent.

Naturally considering the structure of the apparatus, the typical network of somatic cells of vertebrates can only arise from batonettes of the type seen in *Lycastis*. Hirschler's postulate fails to account for the disappearance of

the chromophobic part and if we conceive of the formation of a network from batonettes in which the idiosomic substance is masked such a difficulty does not arise at all. Finally variations in the structure of the network as observed by various cytologists may also be correlated with the variations in the structure of the Golgi bodies as shown in the Plates. As shown diagrammatically below three interesting methods of formation may be probable. In the first type the double-rimmed batonettes by end to end union may give rise to hollow tubular networks as observed by Nassonov and Ludford. In the second, the batonette with no visible idiosomic component may, by a similar process of multiple fusion, give rise to typical networks as figured by Bowen ('26 *a*, *b*, & *c*). Finally there is also the distinct possibility that the vesiculated Golgi bodies found in *Lycastis* where the chromophobic part has been completely reduced as to be absent by multiple folding may singly or by union with one or more similar bodies enlarge and give rise to networks by a mere process of expansion of the connecting chromophil threads. Our conclusions are given below in Text-Fig. 30 in a pictographic manner.



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TEXT-FIG. 30.—A pictographic summary showing the three possible modes of evolution of the Golgi network. (Fig. 14, p. 69, Subramaniam and Gopala Aiyar, 1936*d*.)

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KEY TO LETTERING OF TEXT-FIGURES AND PLATES.

A.—Acrosome; *a.b.*—Acroblast; chb_1.—Chromophobic area between the two chromophilic rims; chb_2.—Chromophobic region in the cup-like area between the two arms of the U.; *chr.*—Chromophilic rims; *g.a.*—Golgi apparatus; $g.b_1$.—Golgi batonette with the idiosome in contact with the cytoplasm; $g.b_2$.—Golgi batonette with the idiosome between the two chromophilic rims; $g.b_{2,x}$.—Golgi batonette showing fusion of the two chromophilic rims leading to the formation of a batonette without any visible idiosomic component; $g.b_3$.—Golgi batonette without any visible idiosomic component; *g.g.*—Golgi granule without any differentiation into chromophilic and chromophobic regions; *g.m.*—Golgi mass; $g.r_2$.—Golgi bodies having the shape of two chromophilic rings one inside the other; *g.r.r.*—Golgi remnant; *g.v.*—Golgi vesicles; $g.v._a$, $g.v._b$, $g.v._c$.—Stages showing the formation of a Golgi batonette by rupture of a Golgi vesicle; *m.*—Mitochondria; $m.f._1$, $m.f._2$, $m.f._3$.—Multiple folding of the wall of the vesicle and also the vesiculated masses that are produced by such a process; *m.g.*—Mitochondrial granules; $g.v._1$, $g.v._2$, $g.v._3$.—Stages in the process of folding of the wall of the Golgi vesicle leading to the formation of double-rimmed batonettes; without a duplex structure; *m.m.*—Macromitosome; *m.t.*—Thread-like mitochondria; *m.v.*—Mitochondrial vesicles; *n.*—Nucleus; *r.g.a.*—Remains of the Golgi apparatus; *s.*—Secretion; *t.g.a.*—Tubular strands of the Golgi apparatus; *v.g.a.*—Golgi apparatus presenting a vesiculated appearance; *V.*—Large vacuoles formed by fusion of a large number of mitochondrial vesicles.

EXPLANATION OF PLATES.

PLATE VII.

FIG. 1.—A portion of *Clibanarius* egg showing Golgi granules, vesicles and batonettes. 15 × 100. Bellows extension 8″. Nassonov. (Original.)

FIG. 2.—A portion of an egg of *Lycastis* with the Golgi vesicles showing various stages of folding of their wall and also the resulting double-rimmed batonettes. 15 × 100. Bellows extension 8″. Modified Champy. (Original.)

PLATE VIII.

FIG. 3.—A portion of an egg of *Lycastis* showing double-rimmed batonettes, batonettes without any chromophobic component and vesiculated masses formed as a result of multiple folding. 15 × 100. Bellows extension 8″. Modified Champy. (Original.)

FIG. 4.—A portion of an egg of Lycastis showing double-rimmed batonettes, batonettes without any chromophobic component and vesiculated masses formed as a result of multiple folding. 15 × 100. Bellows extension 8″. (Original.)

PLATE IX.

FIG. 5.—A portion of an egg of *Lycastis* showing the fusion of the two rims of a double-rimmed batonette. 15 × 100. Bellows extension 8″. Modified Champy. (Original.)

PLATE X.

FIG. 6.—Showing mitochondrial granules and Golgi bodies having the shape of two chromophilic rims one inside the other. 15 × 100. Bellows extension 8″. (Fig. 11, p. 68, Subramaniam and Gopala Aiyar, 1936*d*.)

FIG. 7.—A portion of an egg of *Lycastis* showing various modes of multiple folding of the walls of the vesicle. 15 × 100. Bellows extension 8″. (Original.)

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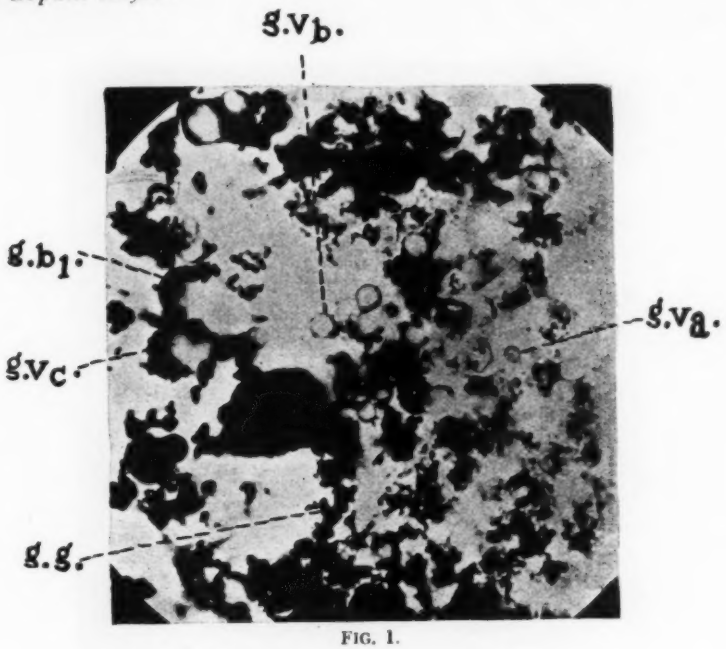
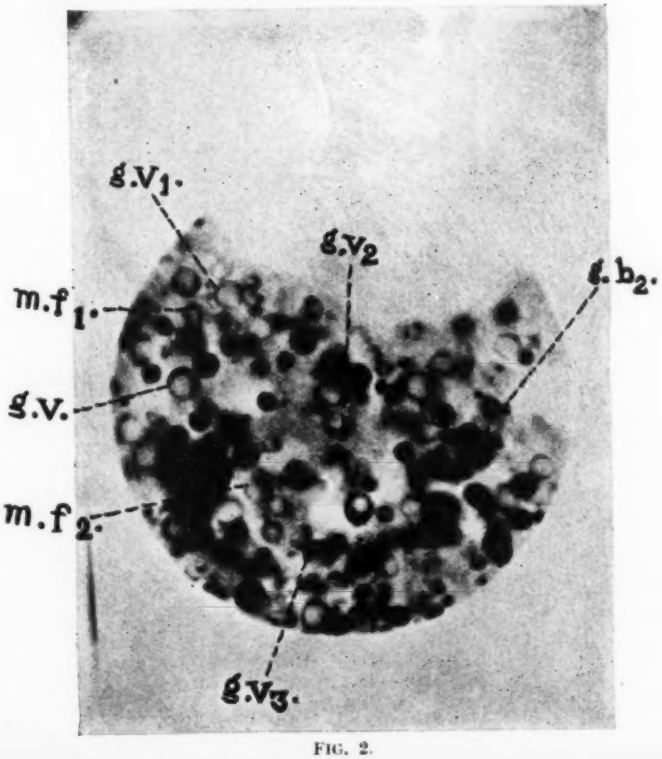


FIG. 1.



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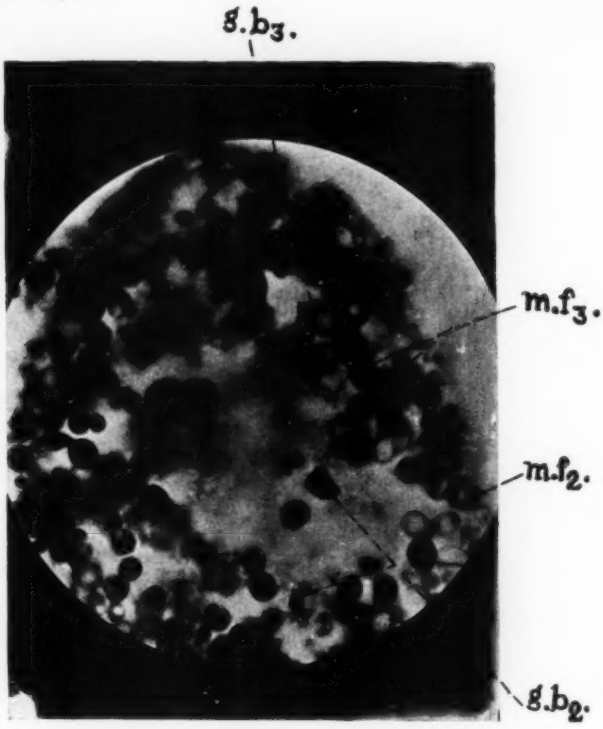
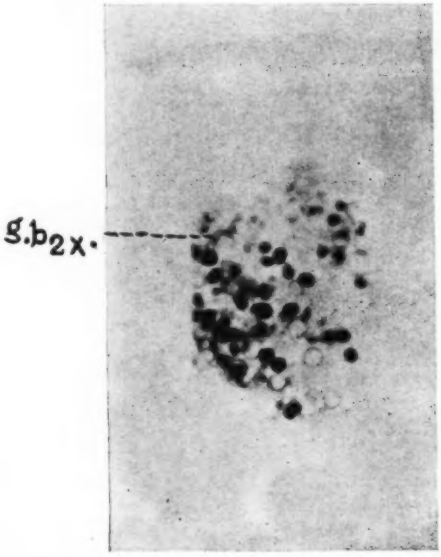


FIG. 3.



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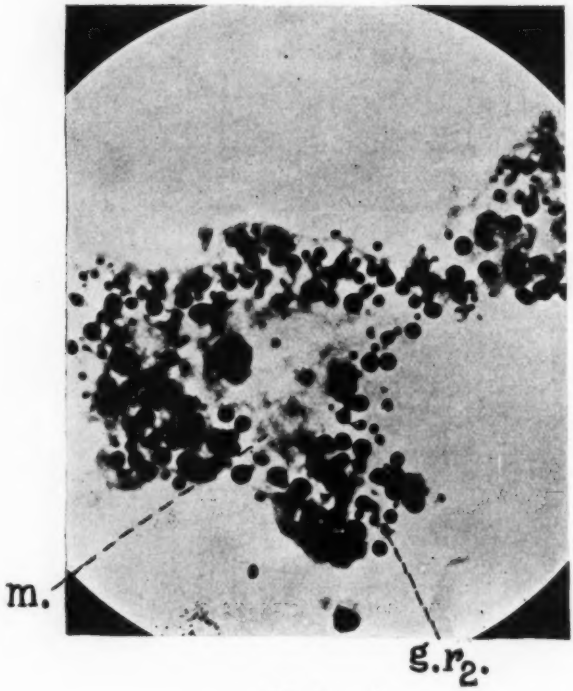


FIG. 5.

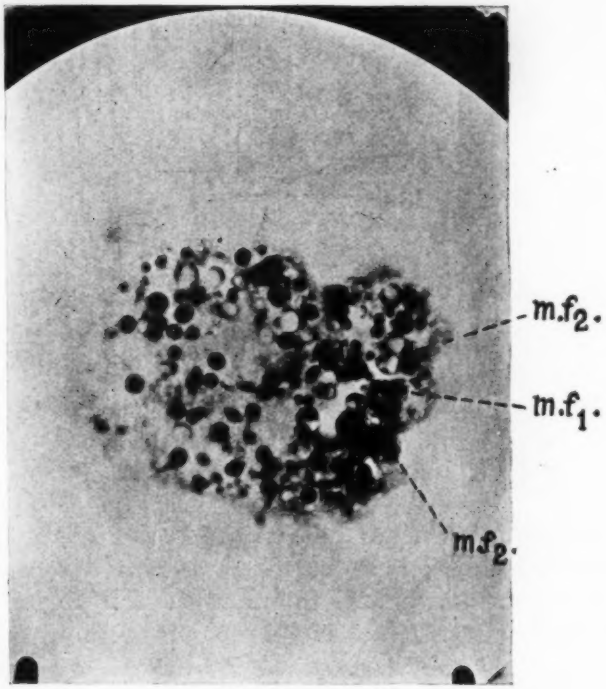


FIG. 6.

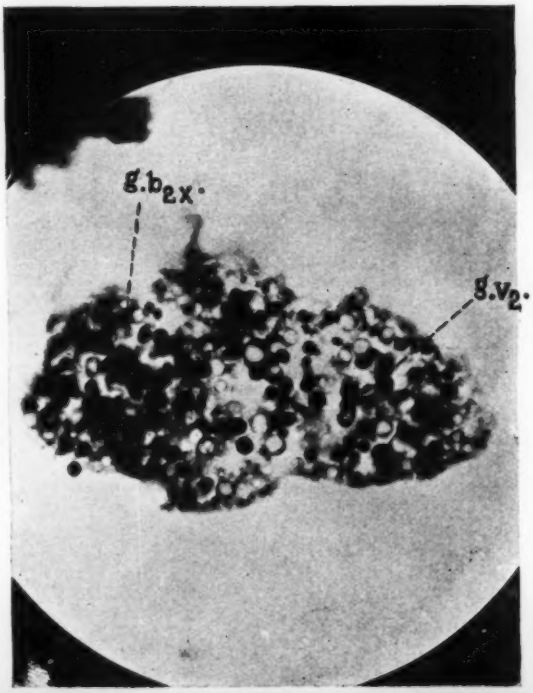
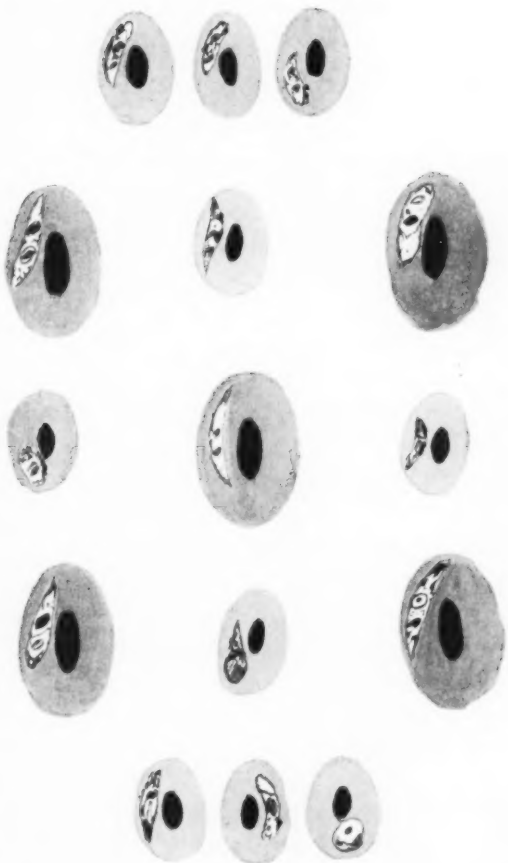


FIG. 7.

Coloured Plate No. VI refers to the article on "On the Classification and Schizogonic Cycle of a Blood Parasite of the Indian Lizard *Calotes versicolor* Daud. Subspecies *Major* Blyth" by Col. I. Froilano de Mello and Caetano Corrêa de Meyrelles appearing in Vol. V, No. 4.



GENETICS OF THE *BANSI* WHEAT OF THE BOMBAY-DECCAN AND A SYNTHETIC KHAPLI—PART II.

By B. S. Kadam, M.Sc. (Cornell),
Crop Botanist to the Government of Bombay, Karjat,
AND
R. K. Kulkarni, B.Ag. (Bombay),
Superintendent, Cereal Breeding Station, Niphad.

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Introduction.

In a previous paper genic analysis of four characters of the two *Bansi* strains, 162 and 167 and of Kala-Khapli 568 was reported, Kadam (1936). It was shown that the red grain colour of Khapli 568 was mono-dominant to the yellow grains of *Bansi* strains. Similarly hairy glumes of Khapli were caused by a dominant gene. The black and red awns, in Khapli 568 and *Bansi* respectively, were the result of two dominant genes, the recessive alleles resulting in white awns. The black awn gene is epistatic to the red awn gene. The latter also causes red glumes and is not influenced by the black awn gene in this region. The genes for pubescence of glumes and black awns were completely linked.

In the present paper genic analysis of three more characters of the *Bansi* wheat and of the Kala-Khapli 568 is presented.

Material and Methods.

The investigation reported in this paper was conducted at the Cereal Breeding Station, Kundewadi, Niphad.

An improved *Bansi* strain, 168, *T. durum*, Desf. var. *hordeiforme*, Körn., was used as a female parent. The strain is a pure line selection from the local *Bansi* wheat. The male parent was Kala-Khapli 568. It is a derivative from a natural cross between Khapli, *T. dicoccum*, and Baxi, *T. durum*, Desf. var. *melanopus*, Kadam (1936).

Crosses between the two types were made during the season of 1932 and F_1 plants raised in 1933. In the succeeding year F_2 plants were raised from 5 F_1 plants. To begin with, there were altogether 172 plants from the 5 F_1 plants, in the F_2 generation. Due to mortality in the seedling stage, only 156 plants reached maturity; and out of these, only 110 plants were obtained which had well filled earheads, the rest either producing shrivelled earheads or very few seeds. In F_3, therefore, there were only 110 families.

The time of flowering and development of anthocyanin pigment was recorded in the breeding plot. The day on which the tip of awns was first seen was reckoned as the day of flowering. The articulation of rachis was studied in the laboratory.

The F_2 segregation shown here represents corrected frequencies based on the breeding behaviour of the 110 F_3 families. For economy of space, the behaviour of only 44 F_3 families is shown. This was the largest population obtained from an F_1 plant.

Previous Work and Results.

Anthocyanin Pigment.

Comparatively few reports have appeared on the inheritance of anthocyanin pigment in wheat. Kajanus (1918) reported red auricles of Mazuola-Americano wheat mono-dominant over the pale reddish auricles of Pearl variety. Jenkin (1925) found a segregation of 3 red : 1 golden straw plant in a natural cross in the Hen Gymro wheat. Goulden, Neatby and Welsh (1928) discovered duplicate genes, P_1 and P_2, for red coleoptile of H-44-22 wheat in a cross with Marquis. The strain H-44-22 is a vulgare derivative from a Marquis × emmer cross. Quisenberry (1931) also detected duplicate genes for coleoptile colour in a cross involving Minhardi and H-44. The latter is related to H-44-32. It is, therefore, very likely that the genes reported by Goulden *et al* and Quisenberry are the same.

In our material, Khapli 568 develops anthocyanin pigment on the plant. The development depends on environmental conditions. The irrigated plants develop a deeper shade than the plants grown under dry conditions. Similarly, the portion of the plant towards the sun develops more colour than the unexposed portion. In *Bansi* 168 no colour develops on the plant except in the auricles and ligule. The colour in these parts develops in later stages. The F_1 plants were coloured like Khapli 568 and in F_2 monogenic segregation of coloured to colourless, like *Bansi* 168, occurred. The frequencies are shown in Table I.

TABLE I.

Segregation of coloured and green plants in the F_2 generation.

Phenotype	Observed	Expected 3 : 1	X^2	P. between
Coloured (A)	89	82·5	2·048	0·20 & 0·10
Green (a)	21	27·5	..	..

Of the 44 F_3 families, 14 bred true to colour and 9 gave green plants like *Bansi* 168 only. The remaining 21 cultures segregated monogenically like the F_2 generation. The expected frequencies on the basis of 1 : 2 : 1 ratio are 11 homozygous coloured : 22 heterozygous coloured : 11 colourless. The X^2 is 1·227 and gives a value of probability between 0·7 and 0·5. The behaviour of the heterozygous families is shown in Table II.

TABLE II.

Behaviour of heterozygous F_2 coloured plants in the F_3 generation.

Culture No.	Observed		Expected 3 : 1		X^2	P. between
	Coloured	Green	Coloured	Green		
1—4—1	8	4	9·00	3·00	0·4444	0·70 & 0·50
„ 4	19	8	20·25	6·75	0·3087	0·70 & 0·50
„ 5	28	2	22·50	7·50	5·3777	0·05 & 0·02
„ 9	14	6	15·00	5·00	0·2666	0·70 & 0·50
„ 15	22	12	25·50	8·50	1·9214	0·20 & 0·10
„ 18	28	8	27·00	9·00	0·1481	0·70
„ 19	18	4	16·50	5·50	0·5453	0·50 & 0·30
„ 22	11	4	11·25	3·75	0·0221	0·90 & 0·80
„ 27	20	4	18·00	6·00	0·8888	0·50 & 0·30
„ 31	14	4	13·50	4·50	0·0740	0·80 & 0·70
„ 33	27	7	25·50	8·50	0·3529	0·70 & 0·50
„ 34	30	8	28·50	9·50	0·3157	0·70 & 0·50
„ 40	16	12	21·00	7·00	4·7618	0·05 & 0·02
„ 51	11	2	9·75	3·25	0·6409	0·50 & 0·30
„ 52	23	7	22·50	7·50	0·0444	0·90 & 0·80
„ 55	30	12	31·50	10·50	0·2856	0·70 & 0·50
„ 57	23	9	24·00	8·00	0·1666	0·70 & 0·50

TABLE II—(*Contd.*).

Culture No.	Observed		Expected 3 : 1		X^2	P. between
	Coloured	Green	Coloured	Green		
1-4-58	21	6	20·25	6·75	0·1108	0·80 & 0·70
,, 62	22	6	21·00	7·00	0·1904	0·70 & 0·50
,, 65	14	5	14·25	4·75	0·0174	0·90 & 0·80
,, 66	21	7	21·00	7·00	0·0000	1·00
					$\Sigma X^2$16·8836	0·70 & 0·50
Total	420	137	417·75	139·25	0·0483	0·90 & 0·80

It will be seen that all the families are in good agreement except 1-4-5 and 1-4-40. But the total X^2, for $n = 20$, shows a value of P. between 0·70 and 0·50, indicating that all the families show essentially similar behaviour.

The anthocyanin gene is designated A.

Brittleness of Rachis.

The two wild wheats, *T. ægiolopides* and *T. dicoccoides* are characterised by very fragile rachis and tight glumes. To a lesser extent fragility of rachis and hard shelling glumes are also found in the cultivated wheats, *T. monococcum*, *T. dicoccum*, and *T. Spelta*. The Indian Khapli wheat, *T. dicoccum*, var. *Ajar*, Mihi, has fragile rachis, like the Abyssinian emmer, with which it is identical, Percival (1921).

Percival (1921) mentions that H. Vilmorin obtained fragile eared forms resembling *T. Spelta*, among the F_2 populations of Blé Seigle (*T. vulgare*) × Poulard Blé Buisson (*T. turgidum*) and Chidham d'automne (*T. vulgare*) × Ismaël (*T. durum*).

Love and Craig (1919) recorded fragileness of rachis dominant over tough condition in crosses involving *T. durum* × *T. dicoccoides* and *T. dicoccoides* × *T. vulgare*, and observed monogenic differences. In the F_2 of a cross between Early Red Chief (*T. vulgare*) × Marouani (*T. durum*) these investigators also recovered 2 plants, out of 113, with fragile rachis similar to those of *dicoccoides*. The segregation indicates the presence of three

duplicate genes. Love and Craig (1924) further crossed the synthetic *dicoccoides* with the true wild emmer and found duplicate genes for brittle rachis.

Vavilov and Jakushkina (1925), in a series of crosses of *T. persicum* with other common wheats with tough rachis, observed marked dominance of fragile rachis of *T. persicum* in F_1. The segregations in subsequent generations indicated that the character is governed by several genes.

In the present case Khapli 568 has fragile rachis while *Bansi* 168 has tough. The F_1 was fragile, but was not so much in degree as the Khapli parent. The F_2 consisted of both types of plants in a 3 : 1 proportion. The frequencies of F_2 are shown in Table III.

TABLE III.

Segregation of rachis nature in the F_2 generation.

Phenotype	Observed	Expected 3 : 1	X^2	P. between
Brittle (T) ..	87	82·5	0·982	0·50 & 0·30
Tough (t) ..	23	27·5	..	..

The agreement of the actual numbers with the theoretical frequencies is good on a monogenic basis.

Out of the 44 families in F_3, 12 showed purity for fragile rachis while the 6 families with tough rachis bred true. The remaining 26 families consisted of both the types of plants. The segregation of the heterozygous families is shown in Table IV.

TABLE IV.

Behaviour of the heterozygous F_2 plants with brittle rachis in the F_3 generation.

Culture No.	Observed		Expected 3 : 1		X^2	P. between
	Brittle	Tough	Brittle	Tough		
1–4–1	9	3	9·00	3·00	0·0000	1·00
„ 13	15	11	19·50	6·50	4·1537	0·05 & 0·02
„ 16	5	5	7·50	2·50	3·3333	0·10 & 0·05
„ 17	23	8	23·25	7·75	0·0106	0·95 & 0·90

TABLE IV—(*Contd.*).

Culture No.	Observed		Expected 3 : 1		X^2	P. between
	Brittle	Tough	Brittle	Tough		
1–4–18	11	4	11·25	3·75	0·0221	0·90 & 0·80
„ 19	16	6	16·50	5·50	0·0605	0·90 & 0·80
„ 22	19	12	23·25	7·75	3·1074	0·10 & 0·05
„ 23	8	3	8·25	2·75	0·0302	0·90 & 0·80
„ 27	18	6	18·00	6·00	0·0000	1·00
„ 30	10	5	11·25	3·75	0·5554	0·50 & 0·30
„ 31	10	2	9·00	3·00	0·4444	0·70 & 0·50
„ 33	27	6	24·75	8·25	0·8181	0·50 & 0·30
„ 34	29	18	35·25	11·75	4·4325	0·05 & 0·02
„ 35	26	12	28·50	9·50	0·8772	0·50 & 0·30
„ 38	10	2	9·00	3·00	0·4444	0·70 & 0·50
„ 42	32	13	33·75	11·25	0·3629	0·70 & 0·50
„ 44	14	6	15·00	5·00	0·2666	0·70 & 0·50
„ 47	19	5	18·00	6·00	0·2221	0·70 & 0·50
„ 52	16	13	21·75	7·25	6·0804	0·02 & 0·01
„ 56	25	10	26·25	8·75	0·2380	0·70 & 0·50
„ 58	20	6	19·50	6·50	0·0512	0·90 & 0·86
„ 59	14	12	19·50	6·50	6·2050	0·02 & 0·01
„ 62	24	4	21·00	7·00	1·7142	0·20 & 0·10
„ 66	21	5	19·50	6·50	0·4612	0·50 & 0·30
					$\Sigma X^2$33·8914	0·10 & 0·05
Total ..	421	177	448·50	149·50	6·7446	> 0·01

It will be seen that most of the families show good agreement with the theoretical frequencies. There are, however, some families which deviate considerably from the expected behaviour. It should be explained that there were considerable difficulties in determining the nature of rachis in certain cases. Instances were found where an earhead would show part of its rachis fragile and the other part tough. Such cases, considered tough in F_2, turned out to be fragile in F_3, indicating difficulty in accurate classification.

In order to determine accurately the nature of the rachis, it is essential that the earheads should be perfectly ripe and dry at harvest. Imperfect drying or immaturity influences fragility and this is most likely to occur in late maturing plants where there are possibilities of harvesting not fully ripe earheads. It will be noted that in families 13, 16, 22, 34, 52 and 59, showing dissorted segregation, the recessive class is in excess in every case. The families 16 and 34 are late maturing, while others segregate for earliness and lateness. Thus, in all of these late flowering plants appear and, therefore, there must have been some cases of imperfect ripening of plants resulting in incorrect diagnosis. Love and Craig (1924) experienced similar difficulties.

In addition to the interference by the above environmental factor, genetic influence is also at work. It was observed that there were varying degrees of brittleness of rachis which defied attempts at classification, but nevertheless connoting undoubted presence of modifying genes. Indeed, in similar crosses it has been possible to build up types which exhibit various intensities of brittleness of rachis.

In two F_3 families, 24 and 61, there were more plants with tough rachis than the brittle plants. These have been excluded, hence there are only 24 families instead of 26 in Table IV.

The genetic grouping of the F_3 families, 12 pure brittle : 26 heterozygous : 6 tough approximates expected frequencies of 11 : 22 : 11, giving a $X^2 = 3 \cdot 090$ and a probability of $0 \cdot 2$. There is thus sufficient evidence to believe that a single major gene causes fragility of rachis and its recessive allele tough rachis. The gene is designated T.

In all cases hard shelling glumes were associated with non-fragile rachis the reverse being the case with tough rachis. The gene T, therefore, causes hard shelling glumes also.

Flowering.

The inheritance of this character has been extensively studied by wheat investigators. Both earliness and lateness have been found to be dominant and either a simple or a complex inheritance has been observed. Farrer (1898)

was the first to observe the inheritance of flowering in wheat. He found F_1 generation intermediate between the parents and a complex segregation in F_2. Studies by Thompson (1918, 1919, 1921) revealed dominance of lateness and transgressive segregation in both directions, the F_2 indicating the presence of many genes. Harrington (1925) noted wide segregation in F_2 of a Mindum × Pentad cross where the parents had the same heading time. Freeman (1918) mentions F_1 and F_2 intermediate, but nearing lateness. In a cross of *durum* × *vulgare* he found F_2 exceeding the range of the late parent. Freeman thinks that 3 or 4 genes may determine heading time. The experience of Bryan and Pressley (1921) was that F_1 was intermediate and F_2 was inclined towards lateness. Stephens (1927), working with three early and three late varieties, came to the same conclusion.

Biffen (1905), Clark (1924), Florell (1924), Clark and Hooker (1926), Crescini (1930) and Nieves (1935) observed dominance of earliness. Biffen obtained 1 : 2 : 1 ratio of early, mid-late and late plants in F_2. Both Florell and Crescini, in one case, report 3 early : 1 late segregation in F_2. The former mentions that minor genes are also present. Clark, and Clark and Hooker record complex segregation in F_2 generation. Aamodt (1927) found an interesting case of two duplicate genes for earliness in a cross of Marquis with a Marquis-Kanred derivative.

Pal'mova and Basova (1934) make the interesting observation that early wheats of southern regions, such as India, Syria, Australia, are dominant in inheritance, whereas those of the steppe may be either dominant or recessive.

In our material *Bansi* 168 is an early strain and has an average heading time of 60 to 65 days, whereas Kala-Khapli 568 heads about a month later. The F_1 plants were intermediate, but definitely inclined towards earliness. As has been stated under "Material and Methods" there were 156 plants in F_2 but only 110 could be grown in F_3 generation. Flowering of both the totals, along with the parents, is given in Table V.

It will be seen that the early parent flowers from 55 to 77 days, most of the flowering taking place from 58 to 64 days. The mean flowering of *Bansi* 168 is 64 days. The late parent, Kala-Khapli, commences heading on the 93rd day and continues upto 107th day, the mean flowering is 99 days. There is a clear cut difference in the flowering of the two parents. The F_2, however, shows an almost continuous range from the 55th to 109th day, with a mean of about 72 days. Most of the plants are grouped towards the left of the table indicating the dominance of earliness. A graphic illustration of flowering is given in Fig. 1.

TABLE V.

Number of days to head, from the day of sowing, of ♀ Bansi 168 *and* ♂ *K.*

| Parents and F_2 | Days to flower from sowing | | |

TABLE V.

from the day of sowing, of ♀ Bansi 168 *and* ♂ *K. K.* 568 *and t*

| Days to flower from sowing | | |

K. 568 *and their* F_2 *generation.*

98	99	100	101	102	103	104	105	106	107	108	109	Total	Mean and S. E.	S. D.	C.V.
												23	64·05 ± 0·7935	5·38	8·39
3	..	..	..	7	..	..	..	..	3			27	99·12 ± 0·5278	3·88	3·91
2	..	..	2	..	..	..	..	..	..	..	1	156	71·87 ± 0·7174	12·67	17·62
												110			

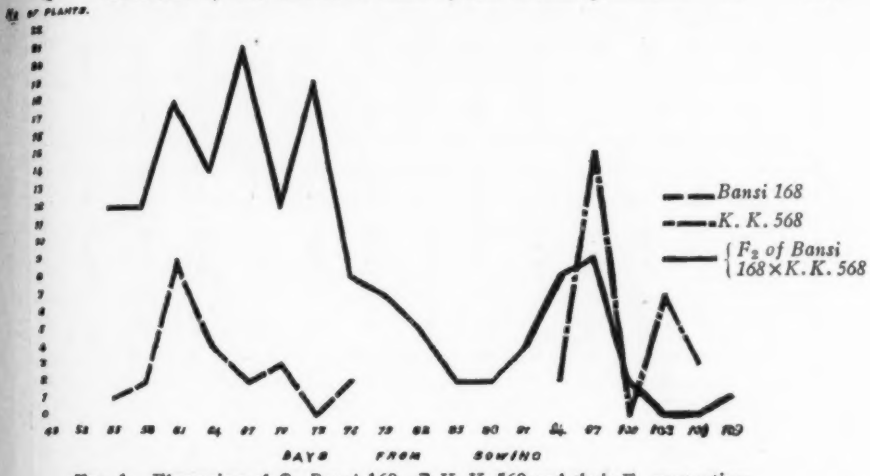

FIG. 1.—Flowering of ♀ *Bansi* 168, ♂ K. K. 568 and their F_2 generation.

If the F_2 population is apportioned in early and late groups by dividing it at the 77th day, the day on which the flowering of the early parent terminates, we obtain the flowering frequencies of early and late plants in the total of 156 F_2 plants and in the 110 plants which were grown in F_3.

TABLE VI.

Early and late plants in 156 F_2 *plants and in the* 110 *plants which were grown in the* F_3 *generation.*

F_2 population of	Observed		Expected 3 : 1		X^2	P. between
	Early (E)	Late (e)	Early	Late		
156 plants ..	116	40	117	39	0·0343	0·90 & 0·80
110 plants ..	86	24	82·5	27·5	0·0594	0·50 & 0·30

It will be seen that on the basis of a monogenic difference both the totals of the F_2 population show satisfactory grouping in early and late plants.

The behaviour of the 110 plants in F_3 clearly indicated that mainly a major gene is concerned in determining flowering with definite indications of the presence of minor genes.

Among the 44 F_3 families, 10 were pure for earliness, 26 were heterozygous and 8 late. The behaviour of these families is shown in Tables VII, VIII and IX respectively.

The mean flowering of the pure early families varies from 61 to about 71 days, while the mean of the *Bansi* parent is 67 days. Thus there are families which are slightly earlier and later than the early parent.

TABLE VII. *Homozygous early flowering F_3 families.*

Culture No.	No. of days required by F_2 plants for heading	Days to flower from sowing																															Total	Mean and S. E.	S. D.	C. V.
		55*	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85				
1-4-9	66	..	..	3	1	3	..	3	..	2	3	..	..	2	..	..	1																18	61·72 ± 0·89	3·81	6·17
,, 21	63	..	..	1	1	1	3	..	..	1	2	1	1	..	..	..	3	2	..	..	1	..	..	..	..	..	..	1	..	..	..	..	18	65·72 ± 1·52	6·44	9·79
,, 24	56	..	1	1	1	4	..	2	1	1	1	..	1	..	..	..	1																14	61·00 ± 1·01	3·78	6·19
,, 27	56	..	4	..	2	5	2	4	2	..	..	2	..	..	..	..	..	..	..	..	1	3											25	62·08 ± 1·22	6·13	9·87
,, 35	62	..	1	1	4	6	7	2	1	4	4	..	1	2	1	..	5																39	62·28 ± 0·66	4·13	6·63
,, 36	57	2	2	3	1	3	3	2	3	..	1	1	2	1	..	1	..	..	..	..	1	1	..	..									27	61·48 ± 0·98	5·11	8·31
,, 44	63	..	..	..	..	..	..	..	..	1	1	..	2	1	2	2	..	..	1	..	1	..	3	..	..	..	..	..	..	..	..	1	15	71·53 ± 1·47	5·71	7·98
,, 47	64	..	..	..	1	2	4	1	2	1	1	1	..	3	2	1	3	1	1	..	..	1	..	..	..	..	..	..	..	..	..	1	26	65·85 ± 1·20	6·15	9·34
,, 51	56	..	..	..	..	..	2	..	1	1	3	1	..	..	..	2	3																13	65·38 ± 1·05	3·78	5·78
,, 55	55	4	1	1	10	8	2	2	4	..	..	1	1	1	1	1	2	..	..	..	..	..	2										41	61·02 ± 0·82	5·27	8·63
Bansi 168 (Parent, early)								2	4	9	14	10	10	7	11	5	15	7	4	4	2	..	1										105	67·20 ± 0·33	3·38	5·03

* First date of flowering was December 24, 1934.

The behaviour of the late families is shown in Table VIII. All the 8 families were distinctly late maturing and show averages

* First date of flowering was December 24, 1934.

The behaviour of the late families is shown in Table VIII. All the 8 families were distinctly late maturing and show averages from nearly 83 to 94 days, while the late parent shows a mean of nearly 83 days. It will be observed that some families are as early as the Kala-Khapli 568 while others show decided shift towards more lateness.

TABLE VIII. *Late flowering F_3 families.*

| Culture No. | No. of days required by F_2 plants for heading | Days to flower from sowing | | |

The flowering behaviour of the 26 heterozygous families is shown in Table IX. It will be observed that all of them show very wide ranges in flowering. All the F_2 parent plants of these families were early. Not a single case of segregation of a late F_2 plant occurs, indicating the recessive nature of lateness. Most of the families did not show any definite segregation. This may be partly due to the smallness of populations, and partly to the presence of minor genes. The fact that the families definitely group themselves in homozygous early, heterozygous early and pure late in the ratio of 1 : 2 : 1 is a definite indication of the presence of a main gene. The gene pair for flowering is designated, Ee.

Intergenic Relations.

The anthocyanin gene A is inherited independently. The unassociated segregation of plant colour and nature of rachis and of plant colour and flowering is shown in Table X.

TABLE X.

Independent segregation of A and T and A and E.

Characters	Phenotypes				X^2	P. between
	AT	At	aT	at		
Plant colour (A) and nature of rachis (T)	70·00	19·00	17·00	4·00	3·034	0·50 & 0·30
	AE	Ae	aE	ae		
Plant colour (A) and flowering (E)	69·00	20·00	17·00	4·00	2·679	0·50 & 0·30
Expected 9 : 3 : 3 : 1	61·87	20·62	20·62	6·87	..	

The gene T, for brittle rachis, is also independent of the gene for flowering. The segregation of T and E is shown in Table XI.

TABLE IX.

Heterozygous early flowering F ... *lies.*

| Culture No. | No. of days required by F_2 plants for heading | Days to flower from sowing | | |

lies.

95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	Total	Mean and S. E.	S. D.	C. V.
																12	77·33 ± 2·79	9·62	12·44
1																27	77·33 ± 1·86	9·65	12·48
..	1															30	78·13 ± 1·65	9·04	11·57
																22	76·70 ± 2·03	9·55	12·41
..	..	..	..	..	1											26	73·92 ± 2·18	11·13	15·06
																21	72·81 ± 1·89	8·66	11·89
																34	74·85 ± 1·71	9·98	13·33
																32	70·00 ± 1·36	7·68	10·97
																38	74·89 ± 1·70	10·45	13·95
1																22	77·27 ± 2·41	11·38	14·73
																15	79·53 ± 2·39	9·27	11·64
..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	11	77·72 ± 4·31	14·52	18·68
..	..	..	..	..	..	1	2									15	80·87 ± 3·41	13·26	16·39
..	..	1	..	..	..	..	..	..	..	..	..	1				16	74·31 ± 3·63	14·49	19·49
																18	69·33 ± 2·09	8·87	12·78
1	..	..	..	3												33	77·78 ± 1·89	10·85	13·94
.	1															12	70·83 ± 3·81	13·19	18·62
1																27	75·74 ± 1·88	9·78	12·91
																18	67·89 ± 1·62	6·88	10·13
..	..	3	..	..	..	..	..	..	..	..	..	..	..	..	1	30	81·23 ± 1·84	10·09	12·42
																33	69·42 ± 1·57	9·01	12·97
																26	68·69 ± 1·82	9·28	13·50
..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	26	75·96 ± 2·49	12·69	16·70
.	1	2	..	..	1	..	1	..	..	..	1	1	1	..	..	28	81·50 ± 2·70	14·26	17·49
																19	73·26 ± 2·31	10·10	13·78
1	..	1	1	1	..	..	..	..	1	..	..	..	1	..	..	27	79·74 ± 2·34	12·21	15·31

TABLE XI.

Independent segregation of T and E.

Characters	Phenotypes				X^2	P. between
	TE	Te	tE	te		
Nature of rachis (T) & flowering (E)	67·00	19·00	20·00	4·00	1·774	0·70 & 0·50
Expected 9 : 3 : 3 : 1	61·87	20·62	20·62	6·87		

Summary.

1. The development of anthocyanin pigment on the plants of Kala-Khapli 568 is caused by a gene A. The recessive allele produces plant like *Bansi* 168, which is colourless except in ligule and auricles.

2. The fragility of rachis in Kala-Khapli is mono-dominant to tough rachis of *Bansi* 168. The action of the major gene, T, is influenced by minor genes.

3. The average flowering period of *Bansi* 168 is from 60 to 65 days. Kala-Khapli 568 flowers about a month later. Earliness in the *Bansi* wheat is caused by a major gene, E, which is modified by subsidiary genes. All the late flowering plants breed true.

4. All the three genes are inherited independently.

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* Originals not seen.

CHLOROPHYLL DEFICIENCIES IN SORGHUM—XANTHA AND PATCHY ALBINO.

By G. N. Rangaswami Ayyangar, B.A., I.A.S.,
AND
T. Venkataramana Reddy, B.Sc., Ag.
(*From the Agricultural Research Institute, Coimbatore.*)

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Many types of chlorophyll deficiencies occur in Sorghum. At Coimbatore, simple monogenic segregations have been obtained between normal chlorophyll, and the following deficiencies, (1) Lethal White, (2) Lethal Virescent White, (3) Lethal Pale, (4) Lingering Lethal Pale, and (5) Surviving Pale (Ayyangar and Ayyar, 1932). In the *Year Book of the Department of Agriculture, United States of America* for 1936, in the chapter on Sorghum Improvement, John H. Martin reporting " the results reported to or obtained by the writer in addition to those found in the literature, " records monogenic segregations between normal chlorophyll and (1) White, (2) Virescent and (3) Yellow. The yellows reported by the American workers are of two categories Y_1y_1 to Y_4y_4 (inclusive) by Karper and Conner (1930) and Y_5y_5 and Y_6y_6 by Quinby and Karper (1934). The literature pertaining to Y_1y_1 and Y_2y_2 (Karper and Conner, 1931) was available to the authors of this article. The other references are not available and it is possible that they are of experiences " reported to or obtained by " Mr. Martin (1936). In this article two fresh experiences in continuation of those already reported from Coimbatore are recorded.

Xantha.

Xantha seedlings were first met with in July 1936 in the progeny of a single head of *Sorghum coreaceum* Snowden from North Rhodesia. The seedling counts gave, green 1,210 and xantha 386. The xantha seedlings could be distinguished by the yellow colour of the seedling leaves, the yellow tint being in the neighbourhood of citron yellow (Ridgway, 1912). They could be spotted five days after sowing. The yellow seedlings begin to die off from the sixth day onwards, the maximum death being between the eighth and tenth days. In rare cases under favourable conditions, xantha seedlings linger to live on to a maximum of two weeks. Twenty-five per cent. of the xantha seedlings had green stripings of sorts on one or both of the first leaf-blades. The leaf-sheaths were free from such striping. The

striping is longitudinal and may vary in width from 0.2 mm. to 3·0 mm. When so striped these seedlings could live a bit longer but never beyond a fortnight.

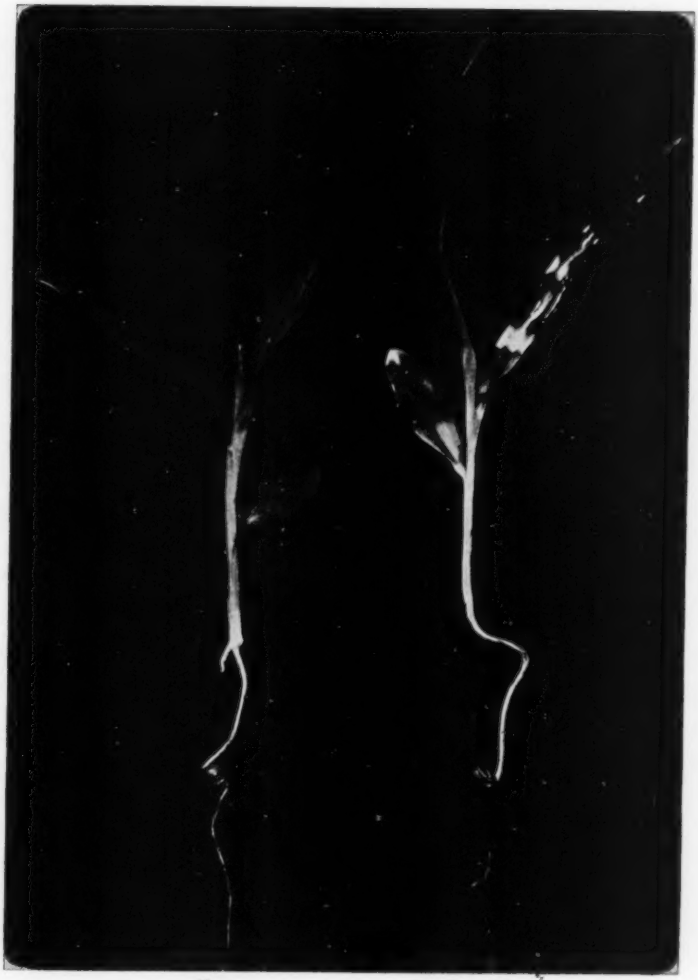
Twenty-five selections were carried forward from the F_2 and of these 15 segregated once again giving the total figures, green 6,401, xantha 2,015. Thirteen out of the 15 segregates threw xantha seedlings with green stripes. The percentage of striped seedlings varied from 19 to 49 with an average of 34·5.

Estimations of chlorophyll and xanthophyll were made by methods similar to those adopted by Demerec (1925) on maize seedlings. The average of six determinations shows that the xantha seedlings had a chlorophyll content of 9·7 per cent. and a xanthophyll content of 97·8 per cent. in comparison with green seedlings. The height of the green seedlings (average of 100, eight days old) was 14 cm. and of xantha 9 cm. The root length of these seedlings was 13·5 cm. in the greens and 8·6 cm. in the xanthas. Hundred seedlings of the green group gave an average weight (four determinations) of 22·7 gm. those of a similar number in the xantha group weighing 12·1 gm.

The gene producing this xantha seedling (which is a simple monogenic recessive to green seedling) has been designated y_x, a symbol in the y_1 series already in use by Karper and others.

Patchy Albino.

The occurrence of lethal albinos that are absolutely white is common in cereals. In sorghum a new experience in which albino seedlings are albinotic in patches has been met with. The whole leaf does not turn white. The leaf remains green but develops erratic patches of chlorophyll-deficient areas on either side of the midrib (*vide* illustration.) These patches tend to be elliptic. They often coalesce across the midrib and give the leaf-blade the appearance of being banded. They are absolutely distinct from being " zebra ". The patches are more mosaic in general outline than definitely delimited like the zebra striping. Patchy albinos can be spotted in seedlings 5 to 6 days old. The seedlings start by being green and develop the patches later on. The chlorophyll in these patches fades to a pale green at first and then to yellow and on to white. This deficiency is manifested in the seedling leaf-sheaths also in a faint manner. These patchy albino seedlings are poor in growth and begin to die from a fortnight to about 20 days after germination. The patchiness being variable some seedlings that are sparsely patchy manage to live a little longer. In the shade and under care with nutrient solutions, a few seedlings could be kept alive for a maximum



Green Patchy Albino

Sorghum Seedlings.

period of two months. The growth at the end of this period is miserable and death is inevitable.

The height of a seedling (average of 100) twelve days old, was 17·3 cm. in the green and 12·8 cm. in the patchy albino. The roots of the green seedling had a length of 15·3 cm. and of the albino 7·6 cm. The average weight of 100 seedlings (four determinations) was 19·1 gm. in the greens and only 6·5 gm. in the patchy albinos.

Segregations for this character have been met with in one South Indian variety belonging to the group of *Sorghum Roxburghii* Stapf. var. *hians* Stapf. and in one race belonging to the group *Sorghum elegans* (Koren) Snowden from the Tanganyika Province of South Africa.

In the South Indian variety the F_2 segregation gave green seedlings 758, patchy albinos 247. Selections from 25 green plants were carried forward and a third generation raised. Of these 25, sixteen segregated once again giving a total F_3 population of 5,630 greens and 1,822 patchy albinos. In the African race the F_2 segregation gave 110 green seedlings and 34 patchy albinos. Four selections were carried forward to an F_3 and of these three segregated giving the following total figures, greens 718, patchy albinos 230. It is noteworthy that this new type of chlorophyll deficiency has been experienced both in an Indian and in an African race of sorghum. The gene responsible for the production of patchy albino has been designated al_p. Al_p results in normal green seedlings.

Summary.

Two new types of chlorophyll deficiencies, *viz.*, xantha and patchy albinos, have been met with in sorghum. Both are lethal. Xantha seedlings are yellow in colour and are deficient in green chlorophyll pigment only. A gene designated y_x is responsible for xantha seedlings. Y_x results in the normal green seedlings. Patchy albinos differ from complete albinos in being greenish in colour with patches of albinotic areas. The gene responsible for the production of these patchy albinos has been designated al_p. Al_p results in normal green seedlings.

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ANTISEPTICS AND ANTHELMINTHICS.

Part III. Pharmacology of Certain Flavones with Special Reference to their Anthelminthic Action.

By Harbhajan Singh Mahal, M.Sc., Ph.D., A.I.C.

(From the Pharmacological Laboratory, Haffkine Institute, and the Royal Institute of Science, Bombay.)

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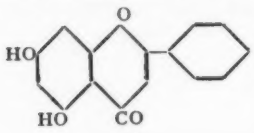
(Communicated by Dr. K. Venkataraman, Ph.D., D.Sc., F.I.C.)

The study of the chemistry of the flavones, the great group of naturally occurring colouring matters, was originated by Piccard[1] who isolated a yellow pigment, chrysin, from the leaf buds of the poplar (*Populus pyramidalis* Salisb., *P. nigra* Linn., *P. monilifera* Ait.), which contained chrysin to the extent of 0.5 per cent. On the basis of degradative and synthetic experiments Kostanecki[2] assigned to chrysin the constitution of a 2-phenyl-5 : 7-dihydroxybenzo-γ-pyrone (I). 2-Phenyl benzo-γ-pyrone or flavone, the parent member of the series, has been found in nature as the meal or farina which occurs on the leaves, stalks and seed capsules of many varieties of *Primula, e.g., P. pulverulenta, P. Japonica* (Muller[3]) ; its synthesis had already been accomplished by Feuerstein and Kostanecki.[4] Since 1873 numerous derivatives of flavone, having different numbers of hydroxyls or methoxyls or both in different positions of the molecule and in the form of glykosides, have been isolated from the roots, stems, barks, fruits, flowers and leaves of various medicinal and other plants ; to cite a few baicalein (II) from the roots of *Scutellaria baicalensis* Georgi, quercetagetin (III) from the flowers of *Tagetes patula*, tricin (IV) from '*Khapli*' wheat and rhamnetin (V) from the husks of *Persian berries*. Due to the wide



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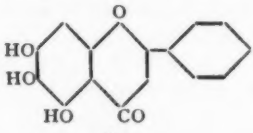

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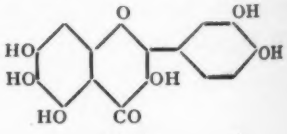

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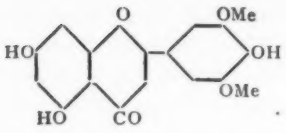
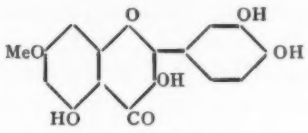
[3] Muller, *J. Chem. Soc.*, 1915, **107**, 875.

[4] Feuerstein and Kostanecki, *Ber.*, 1898, **31**, 1757.



(IV)



(V)

occurrence of the flavones in nature several methods for their synthesis have been developed by various workers, the more important being by Kostanecki and others,[5] Robinson and his pupils,[6] Baker,[7] Mahal and Venkataraman,[8] Algar and Flynn[9] and Mahal, Rai and Venkataraman.[10]

Although so much is known about the chemistry of the flavone group and in spite of their wide occurrence in medicinal plants, *e.g.*, apigenin (VI) and genkwanin (VII) in the Chinese drug ' Yuen-hua ' (Nakao and Tseng[11]), which is reputed to have diuretic and anthelminthic properties and amarbelin in the seeds of *Cuscuta reflexa* Roxb. (Agarwal[12]) also reputed to have anthelminthic properties, very little has been mentioned in literature about the pharmacology of this extensive series of natural and synthetic colouring matters. Koike[13] appears to have been the first to study the pharmacology of the flavones ; he observed that they showed diuresis in normal rabbits and the diuretic action of the polyhydroxyflavones increased with increasing number of hydroxyls. Fukuda,[14] a little later, also confirmed the previous observation of Koike and found further that flavonols and their glykosides are cardiac stimulants, vaso constrictors, and increased the blood pressure.

Ratnagiriswaran, Sehra and Venkataraman[15] isolated a flavone, calycopterin, from the leaves of *Calycopteris floribunda* reputed to have laxative and anthelminthic properties (Nadkarni[16]). On the experimental results of Dr. K. Venkatachalam, Research Officer, Medical College, Madras, who found that calycopterin is toxic to round worms (*Ascaris lumbricoides*) suspended in a mixture of bile with aqueous sodium carbonate approximating to the composition of the intestinal fluid in which the worms reside, they

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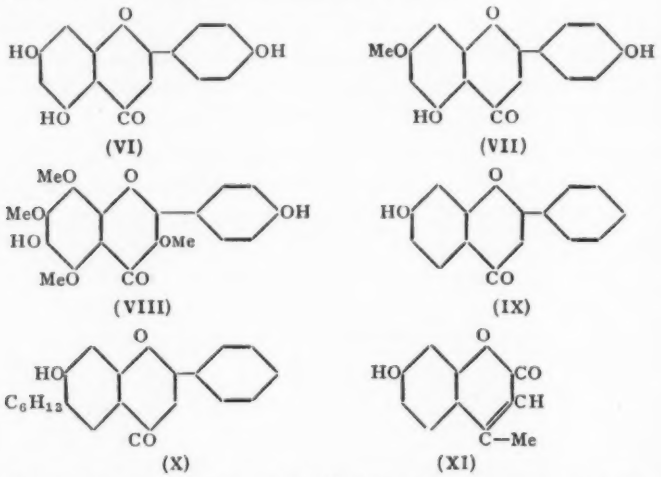
[5] Kostanecki and others, *Ibid.*, 1898, **31**, 1757; 1899, **32**, 2448; 1904, **37**, 3167.
[6] Robinson and his pupils, *J. Chem. Soc.*, 1918, **113**, 859; 1924, **125**, 2193.
[7] Baker, *Ibid.*, 1933, **143**, 1381.
[8] Mahal and Venkataraman, *Ibid.*, 1934, **145**, 1767.
[9] Algar and Flynn, *Proc. Royal Irish Acad.*, 1934, Ser. B, 42, separate issue.
[10] Mahal, Rai and Venkataraman, *J. Chem. Soc.*, 1935, **147**, 866.
[11] Nakao and Tseng, *J. Pharm. Soc. Japan*, 1932, No. 602, 343; 1933, No. 608, 905.
[12] Agarwal, *J. Ind. Chem. Soc.*, 1936, **13**, 531.
[13] Koike, *Folia Pharmacol. Japon.*, 1931, **12**, No. 1, 89, Breviaria, 6.
[14] Fukuda, *Arch. Expt. Path. Pharmakol.*, 1932, **164**, 685.
[15] Ratnagiriswaran, Sehra and Venkataraman, *Biochem. J.*, 1934, **28**, 1964.
[16] Nadkarni, *Indian Materia Medica*, p. 238.

suggested that calycopterin is the anthelminthic constituent of the leaves of *Calycopteris floribunda*. The present author and Venkataraman[17] having further shown that calycopterin is 6 : 4′-dihydroxy-3 : 5 : 7 : 8-tetramethoxy flavone (VIII), the study of the pharmacology, particularly the anthelminthic properties, of flavones has been undertaken.



The flavones that have been studied in the present work are (*a*) chrysin (I) and genkwanin (VII) which are both phloroglucinol derivatives and are therefore of special interest as it is well known that filix derivatives, *e.g.*, male fern, kamala and kausso, which have long been used as specific anthelminthics are phloroglucinol derivatives; (*b*) 7-hydroxyflavone (IX) and 6-hexyl-7-hydroxyflavone (X), derivatives respectively of resorcinol and hexylresorcinol, which are known antiseptics. Along with the study of the pharmacology of benzo-γ-pyrones or flavones, 4-methylumbelliferone (XI), a member of the closely related group of benzo-α-pyrones or coumarins, has also been examined.

The anthelminthic action of the compounds has been studied *in vitro* by (A) directly immersing round-worms (*Ascaris lumbricoides*), tape-worms (*Tœnia serrata*) and leeches (*Hirudo medicinalis*) in different concentrations of the solution of the substances, (B) by the use of Dale and Laidlaw's apparatus.[18] The results obtained in the experiments (A) and (B) show that the compounds do not have any anthelminthic properties; calycopterin itself has also been tried again and has likewise given negative results.

[17] Mahal and Venkataraman, *Curr. Sci.*, 1935, **4**, 311.
[18] Dale and Laidlaw, *J. Pharm. Exper. Therap.*, 1912, **4**, 75.

Although these tests *in vitro* cannot be taken as a conclusive proof that the substances have no anthelminthic action, yet it is a fair indication against their being good anthelminthics. Owing to the very high cost of production of these compounds in sufficient quantities their tests *in vivo* have not been tried.

Other physiological actions of these compounds have also been studied and it has been observed that they inhibit the movements of the isolated rabbit's gut and uterus in concentrations of about 1–140,000 (Plate XIII, Figs. 5, 6 and 7) ; cause depression in the blood pressure, increase the movements of respiration and cause contraction in the uterus of dogs in doses varying from 20–40 mg. per kg. weight of the body of the animals (Plates XIV and XV, Figs. 8, 9, 10, 11 and 12) and in concentrations varying from 1–100,000–500,000 inhibit the beats of an isolated frog's heart (Plate XVI, Figs. 13, 14 and 15).

The antiseptic properties of chrysin, genkwanin and 4-methyl-umbelliferone have been studied on the growth of *B. coli* and it has been observed that chrysin and genkwanin have no effect on the growth of the bacilli, but 4-methylumbelliferone, however, inhibits the growth of the bacilli in 1–1,000 concentration while in concentration 1–10,000 only a slight inhibition has been observed. Germicidal properties of the compounds have been tested by the Rideal Walker's phenol coefficient test and all the compounds are found to have phenol coefficients less than one.

Experimental.

Anthelminthic action of 7-hydroxyflavone (IX), 6-hexyl-7-hydroxyflavone (X), chrysin (I), genkwanin (VII), calycopterin (VIII) and 4-methylumbelliferone (XI)—Method (A).—The compounds being insoluble in water, they were dissolved in minimum amounts of cold N/10 aqueous caustic soda and the stock solutions so obtained were further diluted to the required concentrations by adding the requisite amounts of water, saline or Locke's solution depending upon whether the compounds were to be tried in water, saline or Locke's solution. 6-Hexyl-7-hydroxyflavone being insoluble even in caustic soda solution was used in the form of an emulsion in gum tragacanth. 10 c.c. of each solution of different concentrations was placed in different watch glasses and 2–5 segments of tape-worms previously washed with saline were placed in each. Their activity was constantly watched and when they had completely stopped showing any physical movements they were removed from the watch glasses, washed with saline and put into fresh Locke's solution to see if they could recover their original activity. Leeches and round-worms, for the tests, were dipped into the solution in test-tubes,

plugged loosely with cotton, so that they could not come out of the solution. The results so obtained in a series of experiments with different compounds were as follows :—

TABLE I.

7-Hydroxyflavone, 7-hydroxy-6-hexylflavone, chrysin, genkwanin, calycopterin and 4-methylumbelliferone.

Conc.	1-1,000	1-5,000	1-10,000	1-50,000	1-100,000	Control in distilled water
Leeches	+, +, +	+, +, +, +	+, +, +	+, +, +	+, +, +	Quite active even after 24 hours
Round-worms ..	+, +, +	+, +, +	+, +, +	+, +, +	+, +, +	Do.

TABLE II.

Effect of the compounds on tape-worms.

Conc.	1-1,000	1-5,000	1-10,000	1-50,000	1-100,000	Control in distilled water
7-Hydroxyflavone ..	+, +, +	+, +, +	+, +, +	+, +, +	+, +, +	Active for 45—60 mts.
7-Hydroxy-6-hexyl-flavone ..	+, +, +	+, +, +	+, +, +	+, +, +	+, +, +	Do.
Chrysin	3, 5, 4, 4	7, 8, 10, +	....	13, 15, 17, +	+, +, +, +	Do.
Genkwanin ..	5, +, +, +	10, 12, +, +	....	20, 25, +, +	+, +, +, +	Do.
Calycopterin ..	+, +, +, +	+, +, +, +	....	+, +, +, +	+, +, +, +	Do.
4-Methylumbelli-ferone	8* 3*, +, +	8*, 11*, +, +	10*, +, +, 13*	15*, 15*, +, +	20*, +, +, 20*	Do.

Positive signs (+) in the above tables show that the parasites were as active as in the control experiment; figures in the respective columns show the number of minutes taken by the parasites to die in the particular solution; figures marked (*) show that the parasites stopped their activity during that time and recovered again within five minutes when placed in fresh Locke's solution.

The results given above in Tables I and II were obtained when the solutions of the compounds were prepared in water but when the solutions were prepared in saline or Locke's solution the parasites were as active in the test solutions as in the control experiments.

Method (*B*).—The action of the drugs was studied on tape-worms in Dale and Laidlaw's apparatus.[12] In a number of experiments that were carried out most inconsistent results were obtained. It was observed that in some experiments the inhibitory action of chrysin solution of 1-20,000

dilution was slower than that of 1–40,000 and the worms also regained their original movements when fresh Locke's solution was replaced in the bath where the worm was kept (Plate XII, Figs. 2 and 4) ; in others chrysin solution of 1–20,000 dilution on the contrary activated the worm and 1–5,000 was necessary to stop the movements (Plate XII, Fig. 3), while in still others even 1–5,000 concentration of the drug produced only a slight contraction of the worm (Plate XII, Fig. 1).

Similarly, genkwanin, 7-hydroxyflavone, 6-hexyl-7-hydroxyflavone and 4-methylumbelliferone were also tried but showed no effect on the movements of the worms.

Effect of 7-hydroxyflavone, genkwanin and 4-methylumbelliferone on rabbit's isolated gut and uterine movements.—Dale and Laidlaw's apparatus was used to study the action of these drugs on the intestinal and uterine movements. The rabbit was killed by cutting its throat and allowing it to bleed to death. The small intestines were removed quickly, the intestinal contents washed with tap water and the intestine cut in small bits about 3–4 cm. long. The segments were immersed in Locke's solution and kept in the refrigerator when not in use. One of the segments was fixed in the inner bath of the apparatus with one of its end tied to the air inlet tube and the other to the recording lever by means of fine threads. Solutions of known amounts of each of the substance were then added at a time to the bath containing the segment. The effect of 7-hydroxyflavone and genkwanin on rabbit's gut and of 4-methylumbelliferone is seen in Plate XII, Figs. 5, 6 and 7. The substances stopped the gut and uterine movements in concentrations of 1–140,000, while lesser concentrations produced correspondingly less effects on their movements.

Effect of 7-hydroxyflavone and genkwanin on blood pressure and uterine movements of a dog.—A female pregnant dog (wt. 10 kg.) was anæsthetised with morphia-urethane. The left carotid artery was connected to a mercury manometer through a cannula to record the blood pressure and the right femoral vein was attached to a burette containing normal saline through another cannula. At the same time abdomen of the dog was opened and its uterus was connected by a fine thread to a lever arrangement to record its movements side by side with the blood pressure experiment. Then a concentrated solution of genkwanin dissolved in minimum quantity of 10 per cent. aqueous caustic soda was injected into the animal through the right femoral vein. It was noticed that 100 mg. of the drug clearly produced depression in the blood pressure and contraction in the uterus (Plate XIV, Fig. 8) and the effect was proportional to the amount of the drug injected. Similar results were recorded with 7-hydroxyflavone (Plate XIV, Fig. 9).

Effect of chrysin, genkwanin and 4-methylumbelliferone on blood pressure and respiration of a dog.—The dog was prepared as in the previous experiment but the trachea in this case was connected to a tambour through a cannula to record the movements of respiration. Different amounts of the drugs were injected at a time and their effect recorded. It was found that 200 mg. of each chrysin and 4-methylumbelliferone produced depression in the blood pressure and increased the movements of respiration (Plate XV, Figs. 10 and 12) but in the latter case certain amount of depression in the respiration was also noticed which soon recovered to normal; while the injection of 100 mg. of genkwanin raised the blood pressure for a very short time, again coming to its normal, and an increase in the respiration movements was clearly noticeable (Plate XV, Fig. 11).

Effect of 7-hydroxyflavone, chrysin and genkwanin on the beats of an isolated frog's heart.—A frog was pithed and its chest exposed; two cannulæ were next inserted one in the aorta and the other in the inferior vena cava. All the other blood vessels leading from, and coming to, the heart were ligatured and the heart isolated from the body. A measured quantity of Ringer's solution was then put into the cannula in the inferior vena cava and the aorta cannula was led into the former by means of a small piece of rubber tubing. The whole preparation was fixed on a stand in position, a pinch clip was attached to the apex of the heart and was tied to a heart lever by means of a fine thread and the heart beats were thus recorded on a smoked paper. The Ringer's solution from the circulation was pipetted out as far as possible and the solutions of the substances of different concentrations in Ringer's solution were then added at a time instead. It was observed that 7-hydroxyflavone, chrysin and genkwanin in concentrations 1–200,000, 1–100,000 and 1–500,000 respectively inhibited the action of the heart (Plate XVI, Figs. 13, 14 and 15). A control experiment was also carried out with the maximum amount of caustic soda solution used to dissolve the substance in the above experiments (Plate XVI, Fig. 16) and showed practically no effect on the beats of the heart.

Antiseptic properties of chrysin, genkwanin and 4-methylumbelliferone.—To three different sets of 4 test-tubes each, containing broth culture, was added chrysin, genkwanin and 4-methylumbelliferone so that each one of these compounds had a concentration of 1–1,000, 1–10,000, 1–100,000 and 1–1,000,000 in the four different test-tubes of each set. The tubes were inoculated with a few days' old *B. coli* culture and incubated at 37°C. for 48 hours; a control was carried out along with it by inoculating *B. coli* to pure broth culture. It was found that chrysin and genkwanin had no action on the bacilli, the growth in all the tubes being as profuse as in the control

tube. In the case of 4-methylumbelliferone, however, there was no growth in 1–1,000 concentration and a slight inhibition of growth in 1–10,000 concentration.

Concentrations	1–1,000	1–10,000	1–100,000	1–1,000,000
Chrysin	+ +	+ +	+ +	+ +
Genkwanin	+ +	+ +	+ +	+ +
4-Methylumbelliferone ..	− −	∓ ∓	+ +	+ +

+ = growth ; − = no growth ; ∓ = partial growth.

A control experiment was also carried side by side with these experiments and showed profuse growth of the bacilli.

Germicidal properties of chrysin, genkwanin and 4-methylumbelliferone.—To determine the germicidal properties of the compounds, their Rideal Walker's phenol coefficient was determined in the usual manner. The test was carried out at 18°C. with about 24 hours' old broth culture of *B. typhosus* ; the results thus obtained are tabulated below :—

Time in minutes ..		3	5½	8	10½	13	15½
Phenol	1–105	+	∓	−	−	−	−
Chrysin	1–105	+	+	+	+	+	+
Genkwanin	1–105	+	+	+	+	+	+
4-Methylumbelliferone ..	1–105	+	+	+	+	+	+
„ „ ..	1–525	+	+	+	+	+	+

Control = profuse growth of the bacilli.
+ = profuse growth as in the control.
∓ = partial growth.
− = no growth.

It was therefore evident from the above results that the phenol coefficient of the compounds is less than one.

Summary.

The anthelminthic action of certain flavones has been examined *in vitro*, negative results being obtained. The compounds do not possess any antiseptic or germicidal properties ; they inhibit the movements of isolated

gut and uterus ; intravenous injection of these compounds in dogs causes depression of the blood pressure, contraction of the uterus and increases the movements of respiration ; they inhibit the beats of an isolated frog's heart.

The author wishes to thank Dr. B. B. Dikshit for his guidance in carrying out the present investigation and Dr. K. Venkataraman, Dr. T. S. Wheeler and Lieut.-Col. S. S. Sokhey for their keen interest in the work. The author also wishes to thank the Trustees of the Lady Tata Memorial Fund for the award of a Scholarship to enable him to carry out this investigation.

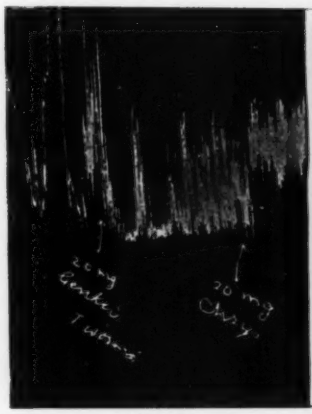


FIG. 1.

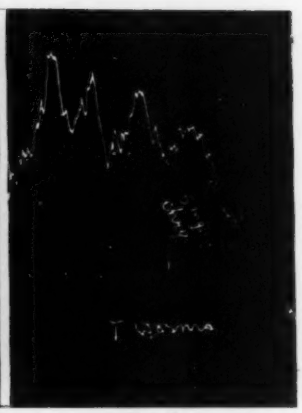


FIG. 2.

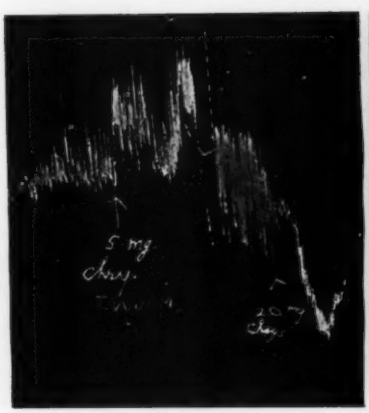


FIG. 3.

FIG. 4.

Note the effect of 1-5,000 genkwanin and of chrysin on the movements of tape-worm Fig. 1, of 1-20,000 chrysin Fig. 2, of 1-20,000 and of 1-5,000 chrysin Fig. 3 and of 1-40,000 chrysin Fig. 4.

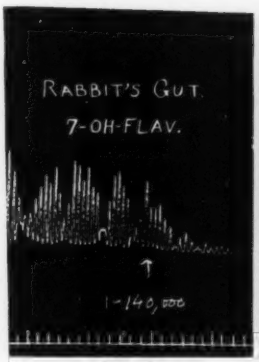


FIG. 5.

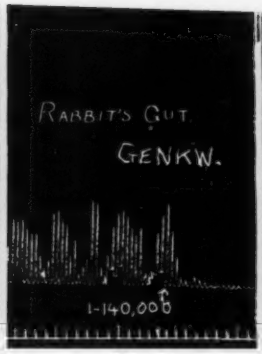


FIG. 6.

FIG. 7.

Note the complete inhibition of the movements of an isolated rabbit's gut caused by 1-140,000 chrysin Fig. 5 and 1-140,000 genkwanin Fig. 6; the effect of 1-14,000 4-methyl-umbelliferone on the movements of an isolated rabbit's uterus is seen in Fig. 7.

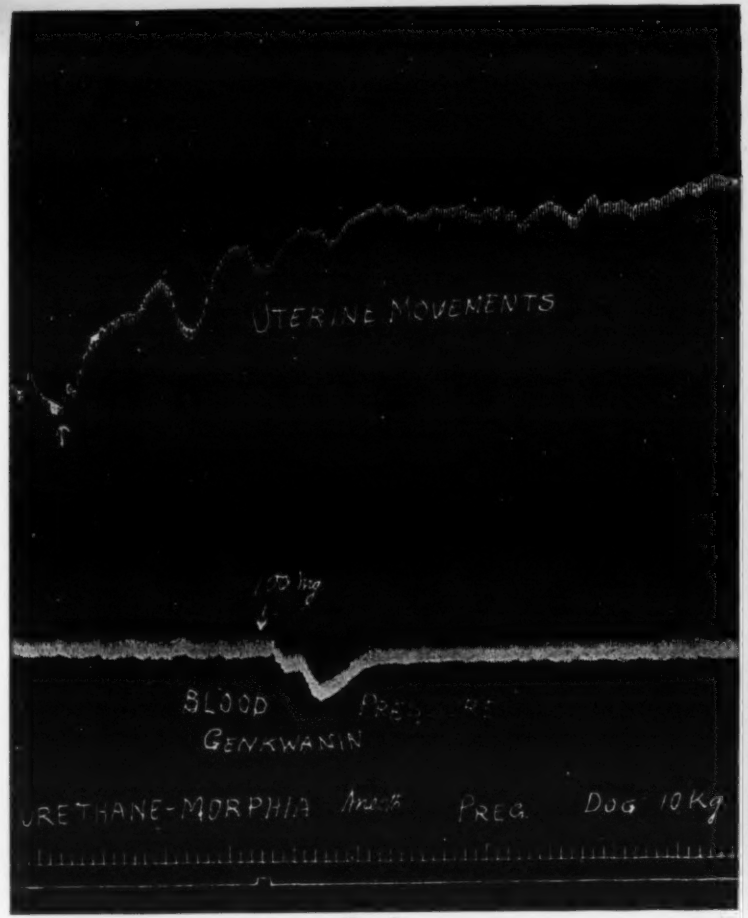


FIG. 8.

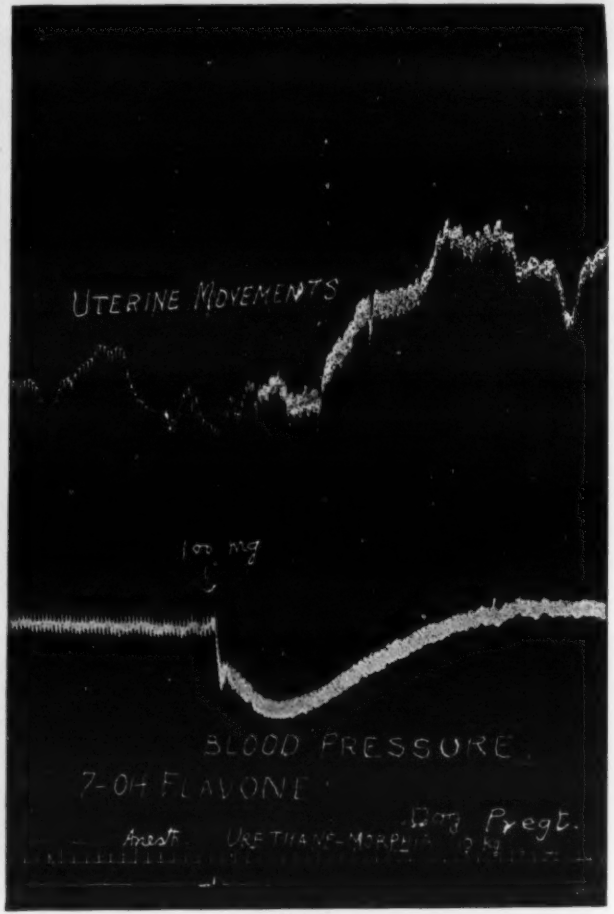


FIG. 9.

♀ Pregnant dog, 10 kg., urethane-morphia anæsthesia. Tracings from above downwards—Uterine movements, Blood pressure, line marker at 10 seconds interval and base line. Note the effect of intravenous injection of 100 mg. of genkwanin Fig. 8 and of 100 mg. of 7-hydroxyflavone Fig. 9 on the uterine movements and blood pressure.

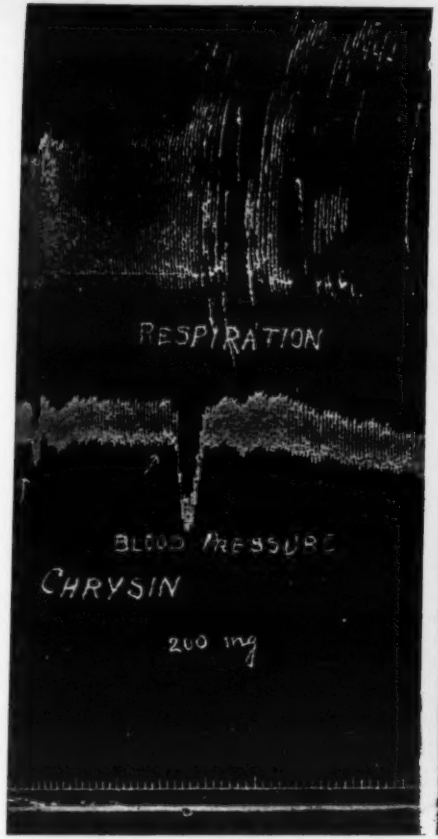


FIG. 10.

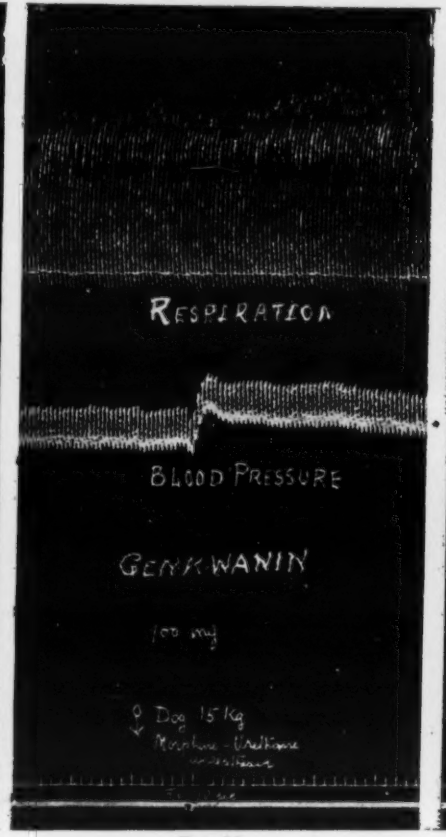


FIG. 11.

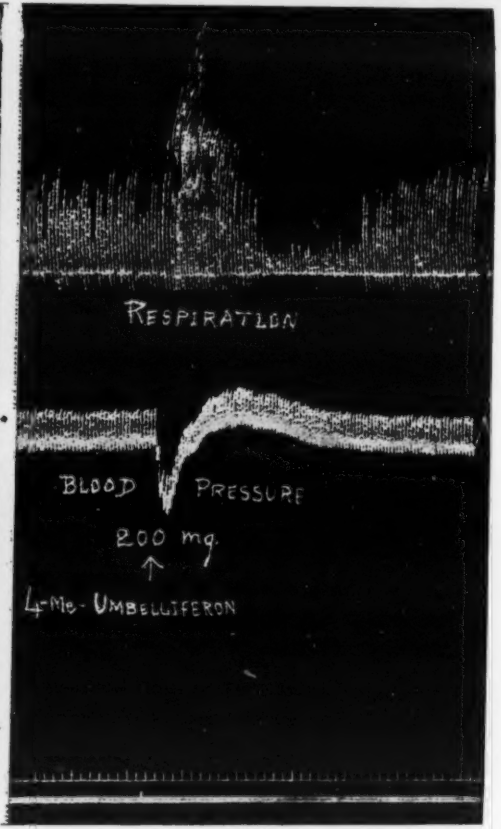


FIG. 12.

♂ Dog, 15 kg., urethane-morphia anæsthesia. Tracings from above downwards—Respiratory movements, Blood pressure, line marker at 10 seconds interval and base line. Note the effect of the intravenous injection of 200 mg. of chrysin Fig. 10, of 100 mg. of genkwanin Fig. 11 and of 200 mg. of 4-methylumbelliferone Fig. 12 on the respiratory movements and blood pressure.

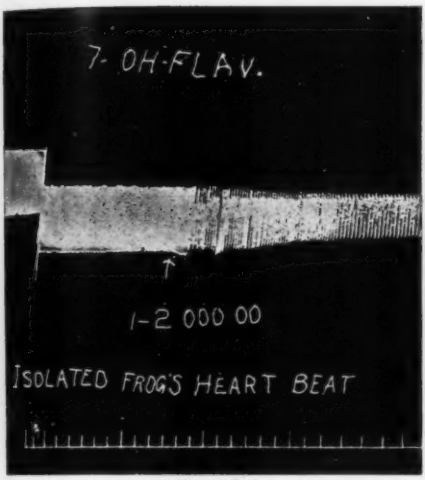


FIG. 13

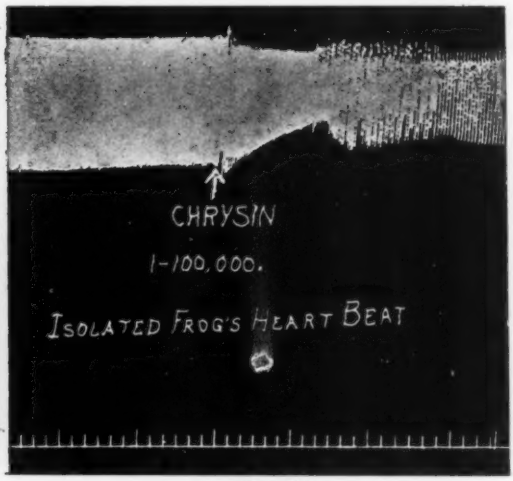


FIG. 14.

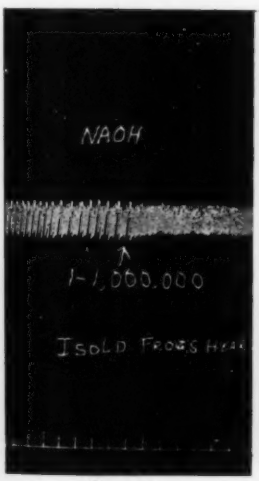


FIG. 16.

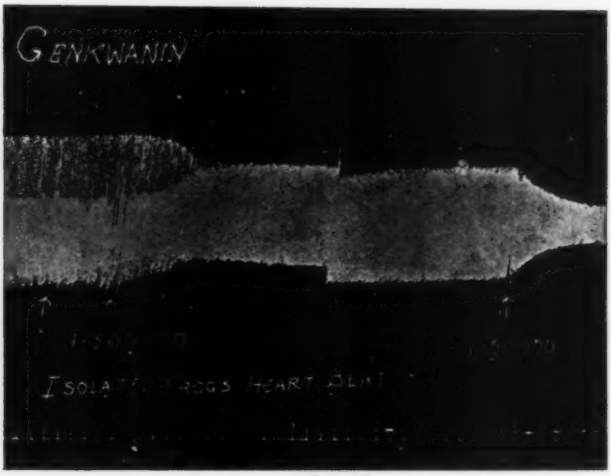


FIG. 15.

Mark the effect of 1-200,000 7-hydroxyflavone Fig. 13, 1-100,000 chrysin Fig. 14, and of 1-500,000 genkwanin Fig. 15 on isolated frog's heart beat ; Fig. 16 shows the effect of 1-1,000,000 NaOH on isolated frog's heart beat.

EMBRYOLOGY OF *ACHYRANTHES ASPERA* LINN.

By L. B. Kajale, M.Sc.

(From the Department of Botany, Benares Hindu University, Benares.)

Received April 3, 1937.

(Communicated by Mr. A. C. Joshi, M.Sc.)

In some previous communications from this Department—Joshi and Rao (1934), Kajale (1935), Joshi (1936), Joshi and Kajale (1937)—contributions have been made to the life-history of two genera of Amarantaceæ, namely, *Digera* and *Alternanthera*. Papers on the first genus have also appeared by Naithani (1933) and Puri and Singh (1935). In the present paper it is proposed to consider a third genus *Achyranthes*. The species selected is *Achyranthes aspera* Linn. The anatomy of the vegetative organs of this plant has been described by Joshi (1931, 1934 and 1937), but so far no paper has appeared dealing with embryological or cytological features.

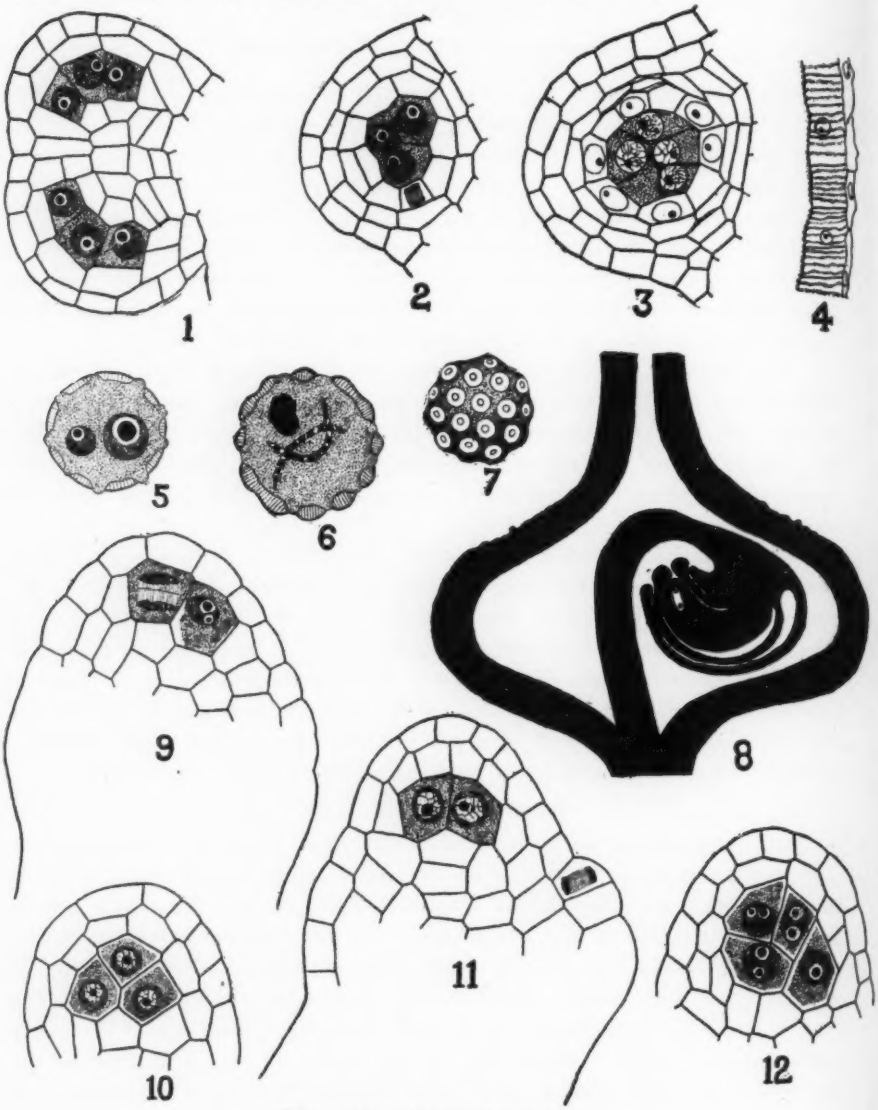
All phases of the life-history of the plant have been studied from the initiation of the flower bud to the formation of the seed. A general resemblance has been observed with the structures found in *Alternanthera* and *Digera*. For this reason, the descriptions given are brief and only points of difference or of sufficient embryological or phylogenetic value are emphasised.

The material was collected locally. It was fixed in Nawaschin's fluid, formalin-acetic-alcohol and some in absolute alcohol alone. An exhaust pump was used while fixing the material. The material fixed in absolute alcohol alone gave as satisfactory results as that fixed in other fluids. There was absolutely no plasmolysis. Usual methods for dehydration and infiltration with paraffin were followed. Sections were cut 5–15 μ thick. The slides were stained with Haidenhain's iron-alum hæmatoxylin and were destained in a solution of picric acid according to Tuan's method.

Organogeny of the Flower.

The various floral parts develop in acropetal succession and the development corresponds to what has been described in *Digera arvensis* (Joshi and Rao, 1934) or *Alternanthera sessilis* (Kajale, 1935). The bracts appear first on the floral axis and are followed by bracteoles. Above the latter, the perianth leaves appear. The stamens arise to begin with as an annular outgrowth and only after some time their primordia become separate. The apex of the floral axis forms the nucellus from the base of which the ovary wall arises as a cup-like structure. As the wall develops, it narrows at the top and forms the hollow style and stigma. The latter develops the

glandular hairs after some time. The further development of the nucellus is accompanied by the formation of a long basal funicle and the differentiation of the two integuments in a basipetal order. The ovule begins to curve just as the primary archesporium differentiates (Fig. 11).



FIGS. 1–12.—*Achyranthes aspera.*

Figs. 1–3, parts of transverse sections of anthers at various stages of development. Fig. 1 shows the cutting off of the primary wall cells. Fig. 3 shows the differentiation of the tapetum and Fig. 2 an intermediate stage. Fig. 4, part of a longitudinal section of a mature anther wall. Fig. 5, 2-nucleate pollen in section. Fig. 6, 3-nucleate pollen in section; the tube nucleus is beginning to degenerate. Fig. 7, a pollen grain in surface view. Fig. 8, longitudinal section of an ovary with an ovule at the 2-nucleate embryo-sac stage. Figs. 9–12, longitudinal sections of the nucellus from different flowers showing a multicellular archesporium and cutting off of the primary wall cells. Figs. 1–3 and 9–12, × 825; Fig. 4, × 670; Figs. 5–7, × 1340; Fig. 8, × 85.

Microsporogenesis.

The primary archesporium in each of the four lobes of an anther in a transverse section consists of 2–4, generally 3, cells. Longitudinal sections of an anther show that it extends from 8–10 cells. It has thus the form of a plate 8–10 cells long and 2–4 cells broad. Thus it is more extensive than in *Digera arvensis*, where there is only one row of archesporial cells in each anther-lobe according to Naithani (1933) and Puri and Singh (1935).

The primary archesporial cells cut off primary wall cells towards the outside and primary sporogenous cells towards the inside (Fig. 1). The former by periclinal divisions form two layers. The outer one below the epidermis forms the fibrous endothecium of the mature anther (Fig. 4), while the inner one divides once more periclinally (Fig. 2). The resulting inner layer towards the sporogenous cells functions as the tapetum, just as has been described by Joshi (1936) in *Rivina* and Joshi and Rao (1936) in *Gisekia*. The layer of cells in between the tapetum and the fibrous endothecium is disorganised very early. The cells of the fibrous endothecium develop punctate markings on their inner walls (Fig. 4), as has been noted in *Chenopodium album* by Bhargava (1936).

The tapetal cells are uninucleate to begin with, but attain the binucleate condition as the pollen-mother cells enter the first reduction division. They show signs of degeneration as the pollen tetrads are being formed and by the time the pollen grains mature they disappear. There is no periplasmodium formation. More than two nuclei were not seen in any tapetal cell. Sometimes two tapetal cells are closely pressed against each other and appear as one cell with 4 nuclei, but a closer examination always reveals the correct nature of such cases.

Simultaneously with the differentiation progressing in the anther wall, the primary sporogenous cells divide twice mitotically to form microspore-mother cells. Each of these cells completes the heterotypic and the homotypic divisions in the normal manner. The four nuclei resulting from these divisions become connected by secondary spindle fibres which disappear as the cytokinesis begins. From the time of the heterotypic division onwards,

the cell wall of the pollen-mother cells becomes progressively gelatinised as reported in *Digera arvensis* by Puri and Singh (1935). This gelatinous sheath is gradually disorganised as the pollen grains separate off from each other.

The cytokinesis takes place by furrowing. The cleavage furrows appear at the periphery of the microspore-mother cells and extend up to the centre to separate the 4 pollen grains. These are generally arranged in a tetrahedral fashion, but sometimes isobilateral arrangement is also seen.

The mature pollen grains differ considerably in size, their diameter varying from 14–20 μ. They are 3-nucleate as in the Centrospermales in general (Fig. 6). The tube nucleus shows an irregular outline and is already beginning to degenerate. The male gametes do not show any distinct sheath of cytoplasm and thus appear to be only naked nuclei. They are very much elongated and are curved in various fashions. They appear banded due to the peculiar distribution of the chromatin.

The exine in sections of the pollen grains shows deeply staining radiating lines, which in sufrace view are seen to form a network (Figs. 6 and 7). In each mesh of the net there is one germ pore. The total number of germ pores in each pollen grain is about 25–30.

The Ovule.

The ovule when fully differentiated shows a form which in many respects appears as campylotropous, but it also shows some characters of anatropy. It may, therefore, be described as ana-campylotropous. There is a long basal funicle and the ovule hangs from its top with the chalazal face placed against one side of the top of the ovary (Fig. 8).

The nucellus is fairly extensive. Its chief feature of interest is the formation of a cap due to the division of epidermal cells. This feature seems to be characteristic of the whole family Amarantaceæ, as it has already been noted in *Digera* (Joshi and Rao, 1934) and *Alternanthera* (Kajale, 1935). The formation of the epidermal cap begins during the development of the megaspores (Figs. 13 and 17). Its thickness varies from 2–3 layers of cells.

The ovule has two integuments. The inner integument is 2 cells thick throughout its length, except at the micropylar end, where it is thicker. It alone takes part in the formation of the micropyle. The outer integument for the major part of its length is 3 cells thick. It is thicker at the micropylar apex, and in the region of the chalaza, it consists of 4–6 layers of cells. During the early stages of embryo-sac development in the chalazal region of the ovule, a prominent air-space is seen between the two integuments.

Another air-space may be seen between the inner integument and the nucellus. During the further growth of the ovule, however, these air-spaces disappear due to the pressure exerted from the inside by the expanding nucellus and no trace of them is seen during the development of the embryo. The presence of similar air-spaces has been recorded in *Chenopodium* by Bhargava (1936), and Maheshwari (1936) writes that Bhargava has also seen it in some other members of the Amarantaceæ, but the names of the plants are not cited.

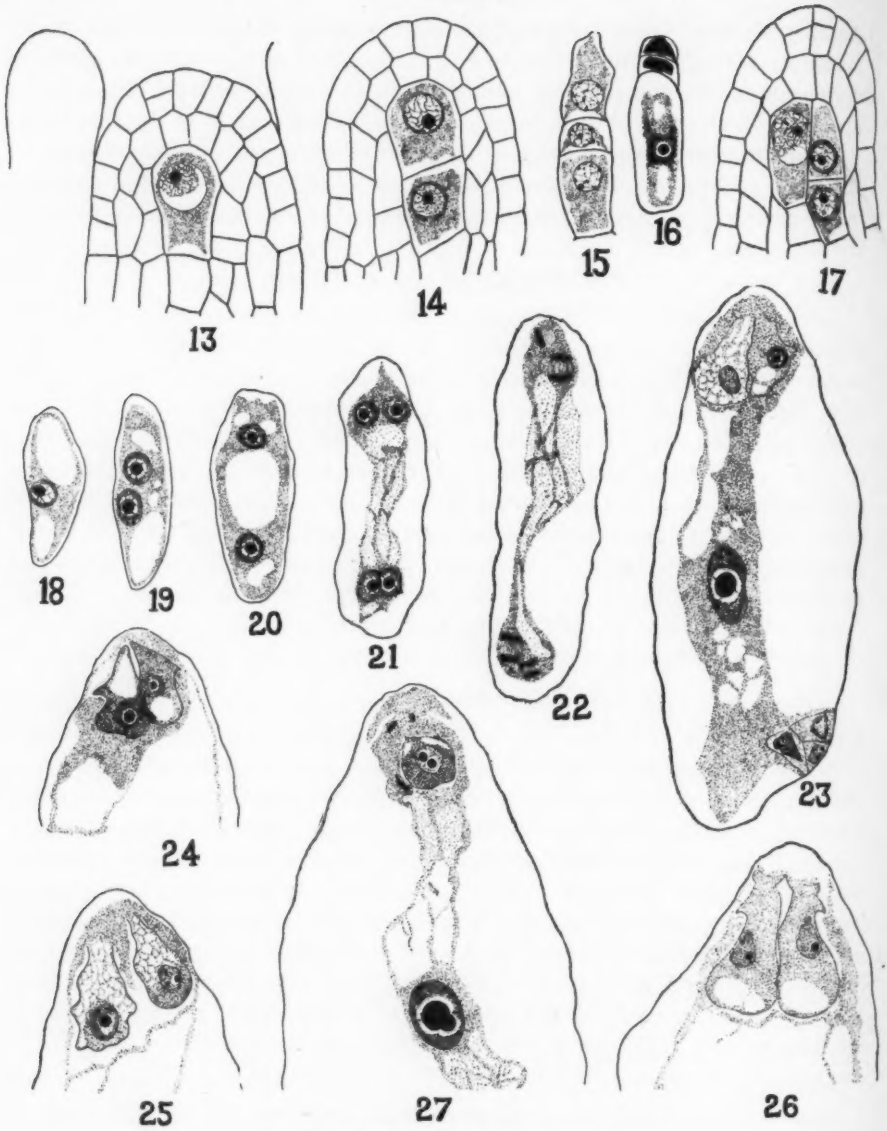
Megasporogenesis and Embryo-sac.

The archesporium in the ovule generally differentiates as a group of 2-5 cells (Fig. 9). All these archesporial cells are not always of hypodermal origin. Sometimes sub-hypodermal archesporial cells are also developed (Fig. 12). Multicellular archesporium is also commonly found in *Digera* (Joshi and Rao, 1934) and *Alternanthera* (Kajale, 1935). Wall cells are cut off only from the hypodermal archesporial cells. Simultaneous differentiation of 2–3 megaspore-mother cells is of frequent occurrence (Figs. 10 and 11), but ultimately only one develops further (Fig. 13). The rest merge into the surrounding tissue. In one case only, two megaspore-mother cells were found to enter the heterotypic division together. This is shown in Fig. 17. The right-hand megaspore-mother cell has completed the heterotypic division, while the nucleus of the second cell is in synizesis.

The primary wall cells by periclinal divisions form about 3–4 layers of parietal tissue.

The megaspore-mother cell gives rise to the dyad in the normal manner. The homotypic division takes place in the chalazal dyad cell only. In this manner, only 3 megaspores are formed (Figs. 15 and 16), as in *Alternanthera sessilis* (Kajale, 1935), *Gompherena decumbens* (Fischer, 1880) and *Amarantus blitum* (Dahlgren, 1916). So far only in *Digera arvensis* in the family Amarantaceæ a complete tetrad of megaspores has been observed, but even in that plant sometimes the micropylar cell of the dyad does not divide any further (Joshi and Rao, 1934). The selection of the megaspore is of the fixed type and the chalazal megaspore alone has been found to develop into the embryo-sac. The other two megaspores degenerate (Fig. 16). The development of the embryo-sac corresponds to the normal type (Figs. 18–23).

The egg is a flask-shaped structure with much vacuolated cytoplasm (Figs. 23 and 25), as noted in *Bœrhaavia diffusa* by Maheshwari (1929). The synergids are hooked. Besides showing the normal structure, they are of interest in rarely showing egg-like vacuolation (Fig. 24), as in *Tamarix* (Joshi and Kajale, 1936) and *Gisekia* (Joshi and Rao, 1936). Full literature



FIGS. 13–27.—*Achyranthes aspera.*

Fig. 13, megaspore-mother cell in synizesis. Fig. 14, a megaspore-mother cell supported by another sporogenous cell. Fig. 15, linear row of three megaspores. Fig. 16, uninucleate embryo-sac and two degenerating megaspores. Fig. 17, an abnormal case where two megaspore-mother cells have simultaneously developed. One is

in the synizesis stage and the second has produced a dyad. Figs. 18–22, various stages in the development of the embryo-sac. Fig. 23, a mature embryo-sac showing an egg-cell, a synergid, secondary nucleus and three antipodals slightly towards one side. Fig. 24, micropylar part of the embryo-sac showing normal and egg-like synergids. Both are hooked. Fig. 25, micropylar part of the embryo-sac with the egg-cell and an egg-like synergid. Fig. 26, the same showing two synergids with 'filiform-apparatus'. Fig. 27, micropylar half of an embryo-sac showing double fertilisation and accessory sperms. × 825.

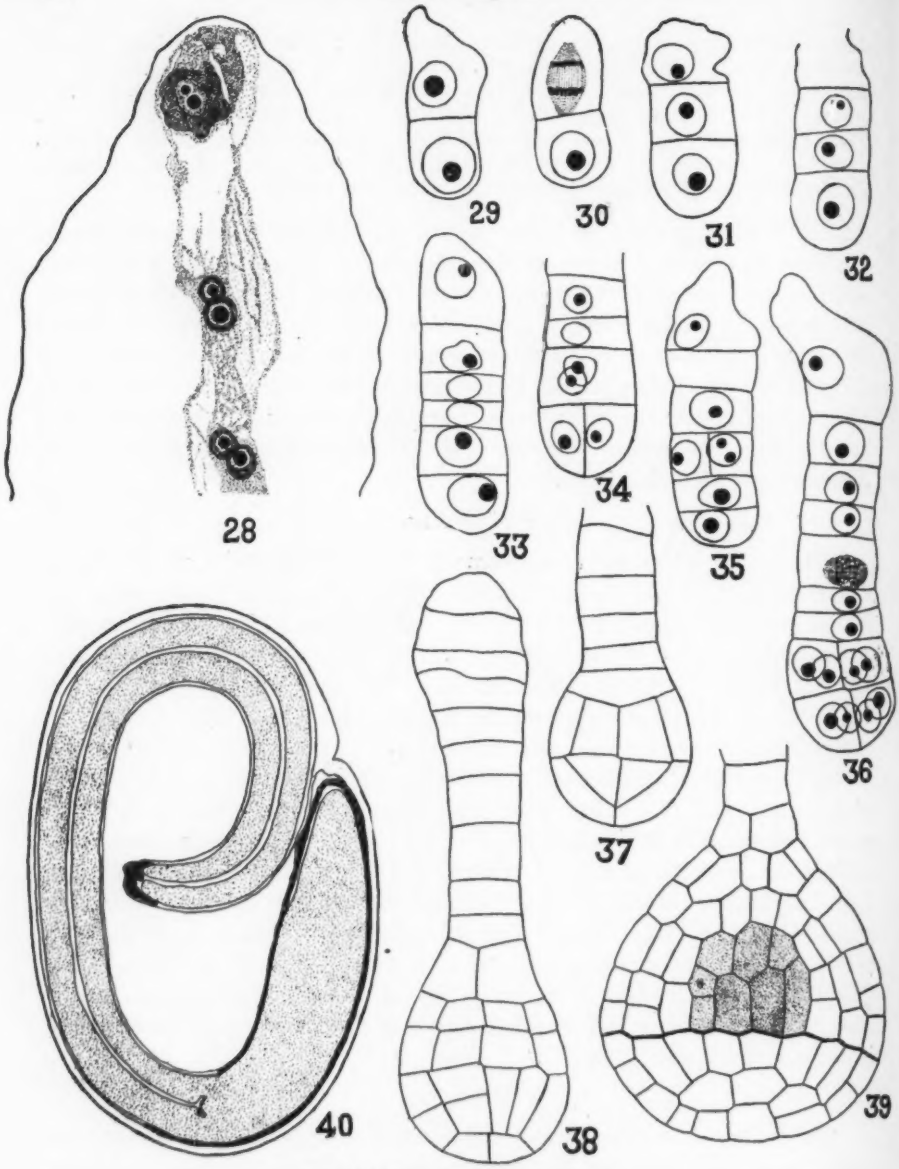
on this subject is cited in these papers. In synergids of one embryo-sac 'filiform apparatus' was clearly seen, though the presence of such apparatus has not been noted in other investigated members of the family (Fig. 26). The polar nuclei fuse before fertilisation. The secondary nucleus always lies in the chalazal half of the embryo-sac (Fig. 23). The antipodals are three in number. They are at first placed at the chalazal extremity of the embryo-sac, but on the beginning of secondary elongation in the embryo-sac the antipodals are left on one side (Fig. 23), as in *Digera* (Joshi, 1936), and *Alternanthera* (Kajale, 1935).

Fertilisation.

The pollen grains are caught among the glandular hairs of the stigma. There they germinate and put forth a pollen-tube, which passes down the hollow style and makes its way to the egg-apparatus through the nucellus and micropylar apex of the embryo-sac. It traverses right up to the base of the egg before discharging its contents. The male gamete concerned in fertilising the egg enters it from the chalazal side (Fig. 27), as noted in *Alternanthera sessilis* (Joshi and Kajale, 1937). The process of fusion is rather slow and sometimes the endosperm nuclei are formed before fusion of male and egg nuclei has been completed (Fig. 28.) The second male gamete fuses with the secondary nucleus. Thus the primary endosperm nucleus is a definite product of triple fusion (Fig. 27). During fertilisation both the gametes change in shape and become more or less spherical. The synergids at this stage are not generally observed. Rarely, however, they persist even after fertilisation, as also noted in *Alternanthera sessilis* (Joshi and Kajale, 1937). The penetration of accessory pollen-tubes into an embryo-sac sometimes can be concluded from the presence of extra sperms in some embryo-sacs (Fig. 27).

Embryo.

The embryo development in *Achyranthes* does not differ in essential points from that described in *Alternanthera* and *Digera* (Joshi and Kajale, 1937), and corresponds to the *Chenopodiaceous*-type of Schnarf (1929). The oospore divides transversally resulting in two cells (Fig. 29). The micropylar cell now divides and a row of 3 cells is formed (Figs. 30 and 31).



FIGS. 28–40.—*Achyranthes aspera.*

Fig. 28, micropylar half of the embryo-sac showing development of the endosperm even before the fusion of the egg-nucleolus and the sperm is complete. Figs. 29–39, various stages in the development of the embryo. Fig. 40, a longitudinal section of a mature seed showing the form of the embryo, a layer of endosperm over the radicle and the perisperm. Figs. 28–38, × 825; Fig. 39, × 670; Fig. 40, × 37.

By further transverse divisions a proembryo of 6–7 cells is organised (Figs. 32 and 33).

The embryo proper results from further differentiation of 3 apical cells. The rest of the cells form a long suspensor of 12 or more cells (Fig. 38). This remains uniseriate throughout its development unlike *Amarantus caudatus* (Woodcock, 1931), *Alternanthera* and *Digera* (Joshi and Kajale, 1937).

Out of the 3 cells which form the embryo proper, the apical cell forms the cotyledons and plumule. The hypocotyl and the greater part of the radicle is developed from the penultimate cell. The third cell which functions as hypophysis completes the root tip.

Occasionally, four cells take part in the development of the embryo proper (Fig. 35). Development in these cases agrees closely with the embryo development in *Chenopodium Bonus-Henricus* described by Souèges (1920).

The first longitudinal wall, in the normal cases, appears in the penultimate cell of the proembryo (Fig. 34). Where 4 cells take part in the development of the embryo, the first longitudinal wall appears in the third cell from the apex (Fig. 35). The differentiation of dermatogen, periblem and plerome (Figs. 37–39) takes place in the same fashion as in *Digera* and *Alternanthera* (Joshi and Kajale, 1937).

The structure of the mature embryo differs slightly from that of *Digera* and *Alternanthera*. In the latter two plants, the embryo ultimately assumes the form of a horse-shoe and surrounds the perisperm. In *Achyranthes*, there is a slightly excessive growth of the cotyledons. They penetrate further into the perisperm than in *Alternanthera* and *Digera* (Fig. 40). Some starch is deposited in all parts of the embryo. It is more abundant in the cotyledons than in the hypocotyl and the radicle. The starch deposition in the primary layers of the embryo is very peculiar. In the region of the cotyledons more starch is deposited in the periblem and dermatogen than in the plerome. In the region of hypocotyl and radicle, it shows just the reverse distribution. It is more abundant in the plerome than in the dermatogen or periblem. In the apical region of the radicle there is no deposition of starch.

Endosperm and Perisperm.

The primary endosperm nucleus divides in a free nuclear fashion and forms about 16 nuclei before the first transverse division of the egg. Wall formation in the endosperm begins at the micropylar end and extends right up to the chalazal extremity. Thus the endosperm becomes cellular throughout its length, as in *Digera*, and differs from that of *Alternanthera sessilis* (Joshi and Kajale, 1937). As the development proceeds, most of the endosperm is utilised by the growing embryo. In the mature seed, it is left as

one or two layers of cells surrounding the radicle in a cap-like fashion (Fig. 40), just as in *Amarantus caudatus* sketched by Woodcock (1931).

A considerable part of the nucellus of the ovule is left behind in the centre of the mature seed as perisperm (Fig. 40), but its form is not exactly spherical or pyriform as in *Amarantus*, *Digera* or *Alternanthera*. The greater growth of the cotyledons in this genus results in a deep furrow on one side in the even outline of the perisperm seen in other plants. Starch is abundantly deposited in the perisperm cells.

Summary.

The anther development of *Achyranthes aspera* agrees with that of *Digera arvensis*, but the archesporium is more extensive. In each anther-lobe, it extends from 8 to 10 cells in length and 2 to 4 cells in breadth. The mature pollen grain is 3-nucleate. The tube nucleus begins to degenerate early. The male gametes are without any sheath of cytoplasm and have banded appearance. The exine is reticulated. There are about 25–30 germ pores, one situated in each mesh of the reticulum.

The ovule is ana-campylotropous and provided with a long funicle. The nucellus shows an epidermal cap. Of the two integuments, the outer is thicker than the inner. The micropyle is formed by the inner integument only. Between the two integuments an air-space is seen in the chalazal region. Sometimes a second air-space is also present between the inner integument and the nucellus. The primary archesporium consists of 2–5 hypodermal or sub-hypodermal cells. A parietal cell is cut off. The megaspore-mother cell forms a row of 3 megaspores. The lowermost develops into the 8-nucleate embryo-sac in the normal manner. The synergids are hooked and a filiform apparatus has been seen in one case. Besides the normal vacuolation, they sometimes show egg-like structure. The secondary nucleus is always situated in the chalazal half of the embryo-sac. The antipodals agree with those of *Digera* and *Alternanthera*.

Fertilisation and embryo development corresponds to that in *Alternanthera* and *Digera*. The penetration of accessory pollen tubes into an embryo-sac is observed in this genus also. The endosperm becomes cellular throughout its length. The form of the mature embryo differs slightly from that of *Digera* or *Alternanthera*. More starch is deposited in the cotyledons than in the hypocotyl. In the cotyledons it is more abundant in the periblem than in the plerome, while in the hypocotyl it is more abundant in the plerome than in the periblem.

In conclusion, I take this opportunity of expressing my thanks to Prof. A. C. Joshi for his kind interest and help in the investigation.

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A CONTRIBUTION TO THE EMBRYOLOGY OF SONNERATIACEAE.

By Jillella Venkateswarlu.

(*From the Department of Botany, Benares Hindu University, Benares.*)

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(Communicated by Mr. A. C. Joshi, M.Sc.)

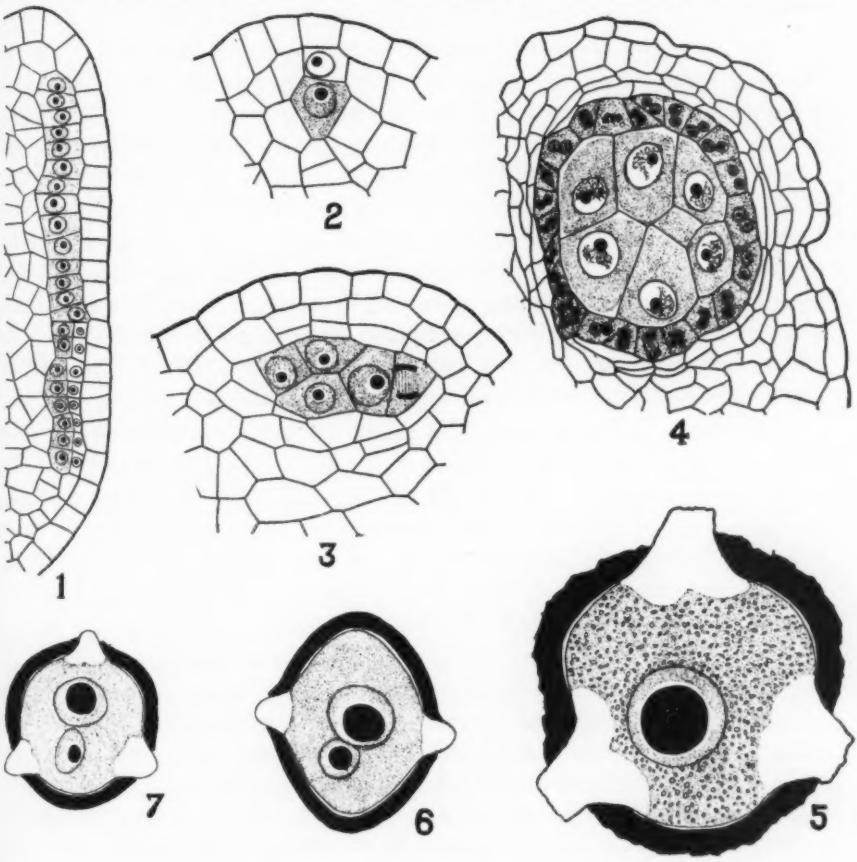
Sonneratiaceæ is a small family of the Myrtifloreæ. According to Engler and Prantl (1898, 1908), it includes four genera, *Sonneratia*, *Duabanga*, *Xenodendron* and *Crypteronia*, comprising about a dozen species. All these are found in the tropics, the monotypic genus *Xenodendron* being confined to New Guinea and the rest being mostly restricted to the Indo-Malayan region. Hutchinson (1926) in his recent system of classification has separated the genus *Crypteronia* into a separate family Crypteroniaceæ and the family Sonneratiaceæ as defined by him includes only three genera. Bentham and Hooker (1862–67), on the other hand, include these genera in the family Lythraceæ, to which there is little doubt that these are very closely related. As the writer had been recently studying the embryology of the family Lythraceæ (Joshi and Venkateswarlu, 1935 *a*, 1935 *b*, 1935 *c*, 1936), it was thought desirable to study the family Sonneratiaceæ also from the comparative point of view and to see how far its embryological features agree with those of the Lythraceæ.

The previous work on this family is limited to an investigation on *Sonneratia apetala* Linn. by Karsten (1891). His observations, however, are very fragmentary and also partly erroneous as pointed out by me in preliminary notes (Venkateswarlu, 1936 *a*, 1936 *b*) relating to the plants described in the present paper.

The present paper deals with the two chief genera of the family, namely, *Duabanga* and *Sonneratia*. The species studied are *Duabanga sonneratioides* Ham., and *Sonneratia apetala* Lamk. The material of the former was collected by me from plants growing in the Royal Botanical Gardens, Sibpur, Calcutta, during the months of April 1933 and May 1935 and was later supplemented by a fresh amount kindly sent by Mr. I. Banerji of Calcutta University. The material of *Sonneratia apetala* was collected by me in April 1936 from plants growing wild in the salt marshes near Calcutta. Material of both the plants was fixed in Nawaschin's fluid and Allen's modified Bouin's fluid. Haidenhain's iron-alum-hæmatoxylin with or without a counter-stain of light green was used for staining the sections.

Development and Structure of Pollen.

The primary archesporium consists of a sub-epidermal row of cells in each of the four lobes of the anther, as may be seen from the longitudinal and transverse sections of young anthers (Figs. 1 and 2). In *Sonneratia apetala* there are about 10 archesporial cells in this row, in *Duabanga sonneratioides* about 20–25 cells. The first division of the primary archesporial



FIGS. 1–7.

Fig. 1, *Duabanga sonneratioides*, longitudinal section of an anther-lobe showing the primary archesporium. Some archesporial cells near the base have cut off parietal cells. Figs. 2–4, *Sonneratia apetala*, transverse sections of various stages in the development of an anther-lobe. Fig. 5, *Sonneratia apetala*, 1-nucleate pollen grain in section along the shorter diameter. Figs. 6–7, *Duabanga sonneratioides*, bi-nucleate pollen grains cut along the longer and the shorter diameters respectively. Figs. 1 & 4, × 450 ; Figs. 2 & 3, × 600; Figs. 5—7, × 1,600.

cells is periclinal and results in an outer layer of primary parietal cells and an inner layer of primary sporogenous cells. The primary parietal cells by periclinal divisions ultimately form 3–4 cells thick wall between the epidermis and the tapetum (Fig. 4). The inner layers of the wall are formed by narrow flattened cells which get crushed with the growth of the tapetum and sub-epidermal wall layer. The primary sporogenous cells undergo a number of divisions in all planes and give rise to a large amount of sporogenous tissue.

The origin of the tapetum could not be followed definitely. Usually it consists of a single layer of cells, but sometimes here and there it is 2-seriate just as was seen in some Lythraceæ (Joshi and Venkateswarlu, 1936). At about the time when pollen-mother cell nuclei enter synizesis, the nuclei of the tapetal cells divide once mitotically, just as in the majority of other angiosperms (Cooper, 1933) and become bi-nucleate.

During the formation of pollen grains, the anthers undergo the usual increase in size and the cells of the epidermis and the sub-epidermal wall layer divide anticlinally to keep pace with the general increase in size of the anther. The sub-epidermal layer in the later stages develops into the fibrous endothecium. The wall layers below it are crushed and the tapetum degenerates without forming any periplasmodium.

The pollen grains separate and round off before the differentiation of intine and exine takes place. The first division of the nucleus in the pollen grains takes place after the full differentiation of intine and exine and the attainment of almost their maximum size. In *Sonneratia apetala*, it takes place just before the dehiscence of the anther. At the time of shedding, the pollen grains are 2-nucleate. No membrane or wall between the vegetative and generative nuclei is seen at this stage.

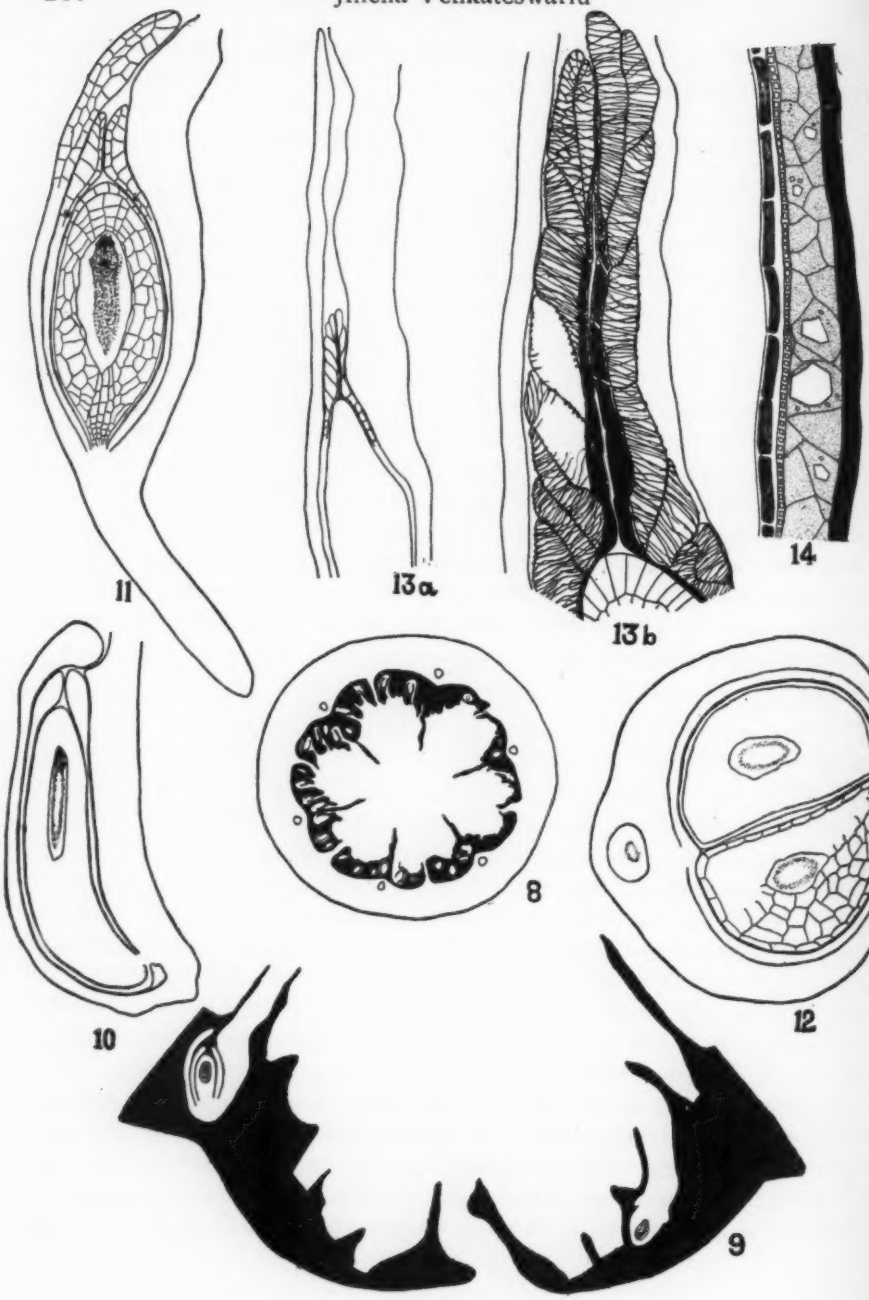
The mature pollen grains are slightly elongated along one diameter. The exine is thick. The surface is smooth in *Duabanga sonneratioides*, while it is rather rugged in the case of *Sonneratia apetala*. Further the pollen grains of the two plants differ remarkably in size. The mature pollen grain of *Duabanga sonneratioides* measures about 16 μ along the shorter diameter and 20 μ along the longer diameter. In *Sonneratia apetala* it measures about 30 μ along the shorter diameter and 35 μ along the longer diameter. There are 3 germ pores in the exine of each pollen grain arranged in an equatorial fashion. Their sections, therefore, along different diameters give rise to different appearances just as in *Ammania baccifera* (Figs. 5–7). The intine protrudes out through the germ pores. The pollen grains of *Sonneratia apetala* have been tested for starch, which is present in considerable amount.

Degenerations in the young sporogenous tissue in the anthers are quite common in *Sonneratia apetala*.

Structure of the Gynœcium and the Ovule.

The ovary is semi-inferior. Usually it is described to be many-celled (10–20-celled) in *Sonneratia* (Engler and Prantl, 1898; Hooker, 1879, Haines, 1922). During the present investigation, in *Sonneratia apetala* it was found to be only 5–6-celled (Fig. 8). Karsten (1891) also describes it to be 5-celled. In *Duabanga sonneratioides*, according to Engler and Prantl (1898), Hooker (1879) and Haines (1922), the ovary is 4–8-celled, but in the material examined by the writer it was found to be often up to 10-celled. In *Sonneratia apetala* the midribs of the carpels, which alternate with the septa, slightly protrude into the loculus of each carpel (Fig. 8), though these are not as prominent as in the ovary of *Lagerstrœmia* (Joshi and Venkateswarlu, 1935 *c*). The placentas are axile and very much enlarged. There is no suggestion of a parietal or sub-basal placentation as described by Rendle (1925). The style is bent upon itself in bud as in some Lythraceæ (*Lawsonia*, *Nesæa*, *Lagerstrœmia*, etc.) and bears a 6–10-lobed, capitate stigma in *Duabanga sonneratioides* and a large, umbrella-shaped stigma in *Sonneratia apetala*.

In *Sonneratia apetala* the ovules are anatropous with a small bend in the chalazal region towards the raphe, a fact also observed by Karsten (1891). They are also slightly flattened towards this side. The funicle is very long and in the chalazal region there is often a small space left between the outer and the inner integuments. The space may be only on one side (Fig. 10) or on both the sides (Karsten, 1891, Fig. 88). The ovules of *Duabanga sonneratioides* are also anatropous in their form, but they are completely cylindrical. They develop long prolongations of their integuments at either end, so that the seed becomes divided into a body or nucleus and two tails (Fig. 11). In *Sonneratia apetala*, there is no such development. In both plants, the ovules are two-integumented and ascending. However, one case was met with in *Sonneratia apetala*, where one of the ovules was found to be in the orthotropous condition at the megaspore-mother cell stage, while all the neighbouring ovules had become anatropous. This ovule also deviates from the rest in only having a single integument (Fig. 9). This exactly coincides with an exceptional case observed in the case of *Nesæa myrtifolia* (Joshi and Venkateswarlu, 1936, Fig. 50). In *Duabanga sonneratioides* occasionally an ovule is found to be descending.



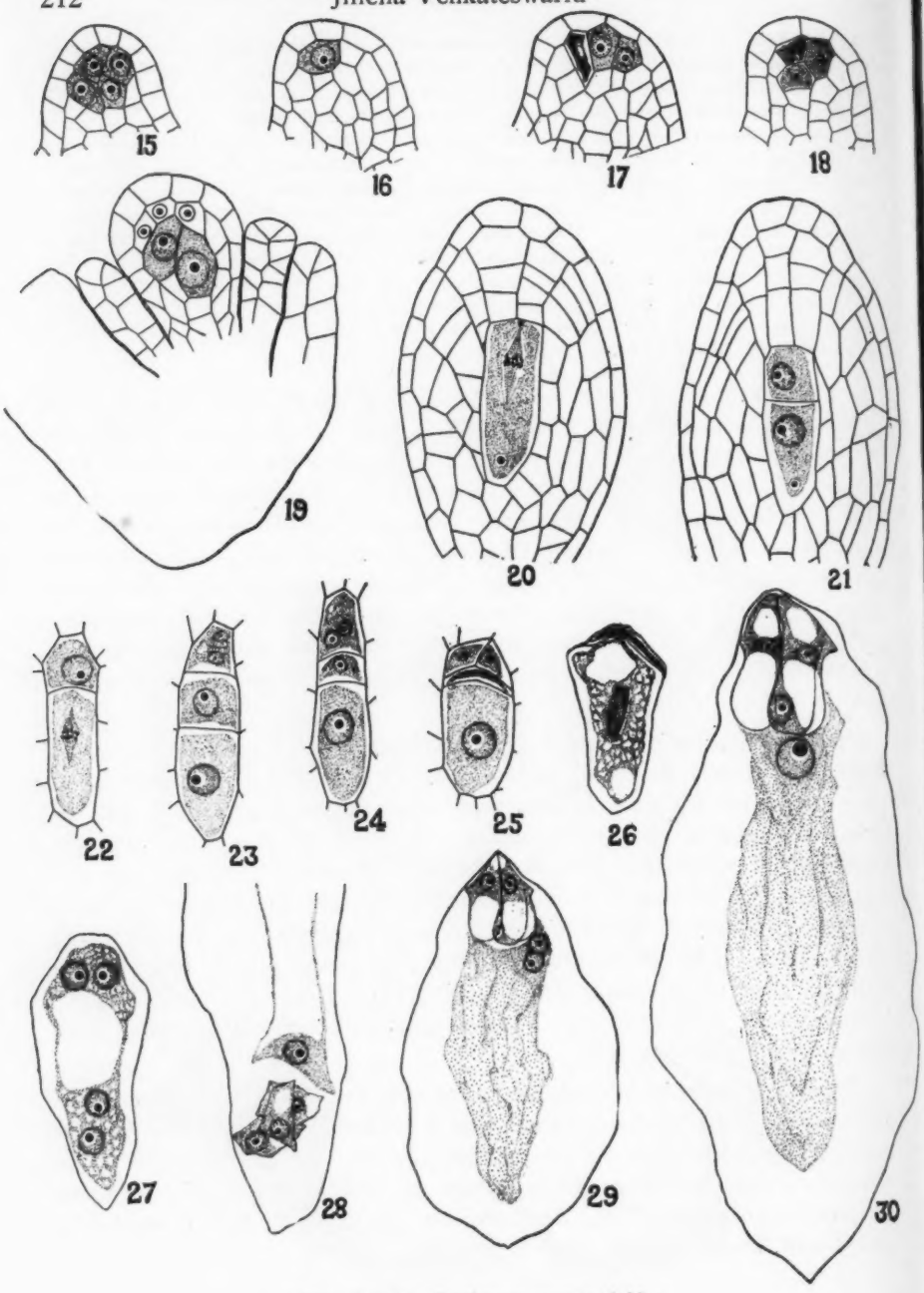
FIGS. 8–14.

Figs. 8–10.—*Sonneratia apetala*. Fig. 8, transverse section of an overy showing 6 loculi and the carpel midribs projecting slightly into the cavities ; Fig. 9, part of Fig. 8 on a higher magnification showing an arthotropous ovule besides a normal one ; Fig. 10, longitudinal section of an ovule at the mature embryo-sac stage showing its form, micropyle and the air space between the two integuments near the chalaza. Figs. 11–14.—*Duabanga sonneratioides*. Fig. 11, longitudinal section of an ovule, showing the form, micropyle, nucellus, conducting strand and prolongations of the integuments ; Fig. 12, transverse section of an ovule showing two nucelli within common inner and outer integuments; Fig. 13*a*, longitudinal section of the upper part of an old ovule showing the long, straight micropyle; Fig. 13*b*, the micropylar part of Fig. 13*a* at a higher magnification showing the spiral and raticulate thickenings of the cells composing the inner integument ; Fig. 14, part of a longitudinal section of the seed-coat from the side of a seed. Fig. 8, × 12 ; Figs. 9 & 13*a*, × 64 ; Fig. 10, × 36 ; Fig. 11, × 160 ; Figs. 12 & 13*b*, × 360 ; Fig. 14, × 640.

The micropyle is formed by both the integuments, as in the Lythraceæ (Joshi and Venkateswarlu, 1935 *b*, 1935 *c*, 1936). In *Sonneratia apetala*, the micropylar canal formed by the inner integument is not in a line with that formed by the outer. Thus the micropyle becomes somewhat zig-zag (Fig. 10), just as in the Lythraceæ (Joshi and Venkateswarlu, 1936). In the case of *Duabanga sonneratioides*, the micropylar canal, though slightly curved in the early stages, is long and perfectly straight in the later stages (Figs. 11 and 13*a*). Both the integuments about the time of fertilisation are 2 cells thick, except at the micropyle, where the outer integument is 3 cells thick. Their development and structure in the seed is described later.

The nucellus in the ovules ready for fertilisation consists of about 5–6 layers of cells above the embryo-sac. On the sides of the embryo-sac there are about 3–4 cells in *Duabanga sonneratioides* and 7 or 8 cells in *Sonneratia apetala*. Below the embryo-sac there are about 8–10 cells in *Duabanga sonneratioides* and 40–50 cells in *Sonneratia apetala*. In this region a strand of elongated cells with rich protoplasmic contents is differentiated in *Duabanga sonneratioides* and is quite similar to the conducting strand described in the Lythraceæ. Such a strand of cells is not differentiated in *Sonneratia apetala*. On the other hand, there takes place a great increase in the number of cells in this region and the ovule becomes bent towards the raphe (Fig. 10).

Two nucelli within an ovule have been observed in one case in *Duabanga sonneratioides*. In this exceptional case both the inner and outer integuments were common for the two nucelli (Fig. 12). Similar cases are quite common in the Lythraceæ and have been seen in *Cuphea lanceolata, Cuphea petiolata* (Mauritzon, 1934), *Lagerstræmia indica* and *Nesæa myrtifolia* (Joshi and Venkateswarlu, 1935 *c*, 1936).



FIGS. 15–30. *Duabanga sonneratioides.*

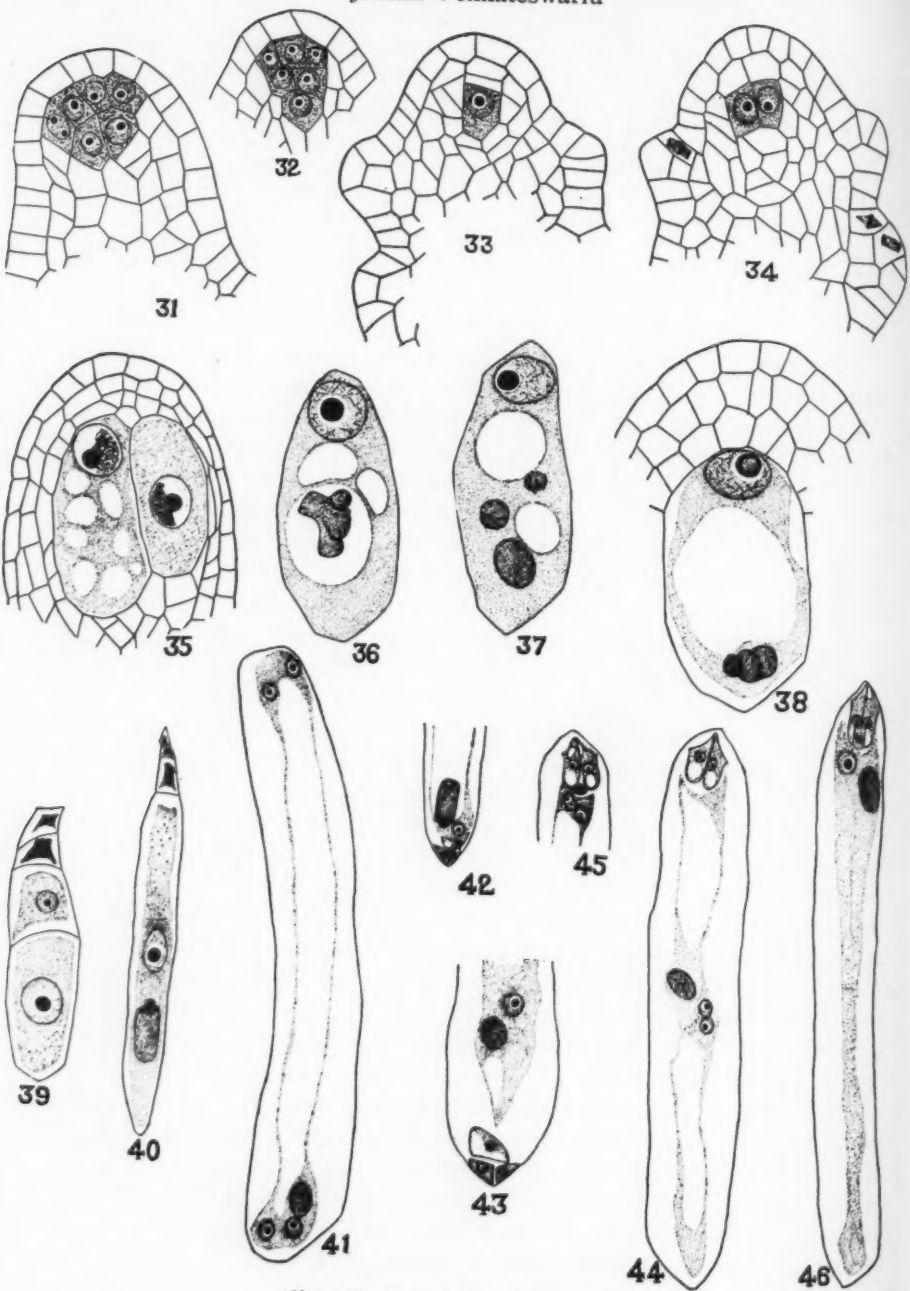
Figs. 15–18, primary archesporium ; Fig. 19, two megaspore-mother cells in one ovule; Fig. 20, a nucellus with a megaspore-mother cell in the heterotypic division (metaphase) and also showing the presence of extra-nuclear karyotin bodies: Fig. 21, a nucellus showing a dyad, the upper cell being small and the lower being bigger; Fig. 22, a dyad with its lower cell dividing ; Fig. 23, a dyad with the upper cell dividing ; the lower cell has already completed its division; Fig. 24, a row of 3 megaspores, the upper being 2-nucleate; Fig. 25, a T-shaped tetrad of megaspores ; Fig. 26, 1-nucleate embryo-sac with the nucleus in telophase; Fig. 27, 4-nucleate embryo-sac; Fig. 28, lower part of an embryo-sac showing the antipodal cells and the lower polar nucleus; Fig. 29, embryo-sac after the disappearance of the antipodals; Fig. 30, a mature embryo-sac; Figs. 15–21 and 26–30, × 640; Figs. 22–25, × 720.

Development and Structure of the Embryo-sac.

The number of primary archesporial cells in an ovule is generally more than one, but occasionally in *Duabanga sonneratioides* only one (Fig. 16). These cells are mostly hypodermal in origin, but occasionally sub-hypodermal cells are also found with archesporial characters (Figs. 15, 17, 18, 31 and 32). As in the Lythraceæ (Joshi and Venkateswarlu, 1935 *b*, 1935 *c*, 1936), usually only one archesporial cell develops further, cuts off a parietal cell by a periclinal division and becomes the megaspore-mother cell. The rest of the archesporial cells merge into the ordinary nucellar cells, but in *Duabanga sonneratioides* the non-functional archesporial cells sometimes begin to degenerate instead of merging into the nucellus. Fig. 17 illustrates an ovule with a sub-hypodermal group of 3 archesporial cells, out of which one cell (on the left) is seen to degenerate. Fig. 18 shows a case of an ovule showing two hypodermal and two sub-hypodermal archesporial cells out of which the former are seen to degenerate and the latter develop further. This feature has not been observed in any Lythraceæ.

Exceptions to the above usual condition are frequently met with as in the Lythraceæ. Fig. 19 shows a case of two megaspore-mother cells lying side by side in an ovule of *Duabanga sonneratioides*. Fig. 34 shows a similar case in *Sonneratia apetala*. In both the above cases, it seems that two adjacent hypodermal archesporial cells have developed and have cut off parietal cells. Fig. 35 illustrates a case of two advanced megaspore-mother cells with their nuclei in synizesis in an ovule of *Sonneratia apetala*. However, no instances of double embryo-sacs as in some Lythraceæ (Joshi and Venkateswarlu, 1935 *b*, 1935 *c*) have been observed in these plants.

The primary parietal cell first divides either anticlinally or periclinally (Figs. 19 and 33). A number of anticlinal and periclinal divisions follow and make the parietal tissue 4–5 cells thick in the fully formed ovules. No tapetal layer is found to be formed around the megaspore-mother cell. Only in *Duabanga sonneratioides*, as mentioned previously, a conducting strand is developed in the chalazal region of the nucellus.



FIGS. 31—46. *Sonneratia apetala.*

Figs. 31–32, primary archesporium; Fig. 33, megaspore-mother cell; Figs. 34 & 35, two megaspore-mother cells lying side by side; Figs. 35–38, advanced megaspore-mother cells showing the presence of the extra bodies in them; Figs. 39 & 40, linear tetrad of megaspores at different developemental stages. Fig. 41, 4-nucleate embryo-sac showing the extra body; Figs. 42 & 43, lower parts of two young embryo-sacs at different stages showing the antipodals, lower polar-nucleus and the extra body; Fig. 44, embryo-sac after the disappearance of the antipodals with the extra body; Fig. 45, micropylar part of an embryo-sac showing egg-apparatus and the two still unfused polar nuclei near it; Fig. 46, a mature embryo-sac. Figs. 31–39, × 640; Figs. 40–46, × 360.

The megaspore-mother cell stage lasts for a long time. In *Sonneratia apetala*, a great increase in size is witnessed in this cell and it crushes to some extent the surrounding cells of the nucellus. The protoplasm becomes also very much vacuolated (Figs. 35–38). The nucleus, when it enters the synizesic stage, is usually found in the micropylar end of the megaspore-mother cell. The heterotypic division also consequently takes place in the micropylar region and results in a small upper dyad and a big lower dyad cell (Figs. 20 and 21). This recalls a similar condition observed in *Woodfordia floribunda*, *Nesæa myrtifolia* (Joshi and Venkateswarlu, 1936) and *Cuphea lanceolata* (Mauritzon, 1934). The tetrad formation has been closely followed in *Duabanga sonneratioides*. The chalazal cell undergoes the homotypic division much earlier than the micropylar dyad cell (Figs. 22–24). The tetrad is generally linear, but sometimes in *Duabanga sonneratioides* it is T-shaped. Rarely it seems that the wall formation between the daughter nuclei of the micropylar dyad cell fails to take place. This gives rise to a row of 3 megaspores, the uppermost being 2-nucleate and the lower two being uni-nucleate (Fig. 24). A similar case was figured by Mauritzon (1934, Fig. 1E) for *Peplis portula*. The chalazal megaspore of the tetrad is always the functional one. It is very much elongated in *Sonneratia apetala*. The other megaspores are always found to degenerate.

The development of the embryo-sac corresponds to the normal type. The antipodals are organised into definite cells (Figs. 28, 42 and 43). These degenerate very early and completely disappear (Figs. 29, 30, 44 and 46) by the time the polar nuclei come together as in most Lythraceæ. The polar nuclei meet each other about the middle of the embryo-sac, move upwards and take their position near the egg-apparatus (Figs. 29, 44 and 45). Ultimately they fuse in this position to form the secondary nucleus which lies just below the egg-apparatus (Figs. 30 and 46). The movements and behaviour of the polar nuclei correspond to that exhibited by members of the Lythraceæ. The cells of the egg-apparatus also resemble those of Lythraceæ. The mature synergids bear prominent hooks and a small vacuole in their apices besides the chalazal vacuole characteristic for angiosperms in general.

The egg is flask-shaped, with a large micropylar vacuole and a small nucleus lying in the scanty cytoplasm at its chalazal end.

The form and size of the embryo-sac differs in the two plants. It is broader at the chalazal end and narrower towards the micropylar end in *Duabanga sonneratioides* (Fig. 30). In *Sonneratia apetala*, it is greatly elongated and is equally broad throughout its length (Figs. 44 and 46). At the time of fertilisation, it measures about 165 μ long and about 70 μ broad (at the broadest point) in *Duabanga sonneratioides*, and about 250 μ long and about 40 μ broad in *Sonneratia apetala*.

One very interesting fact about the embryo-sac of *Sonneratia apetala* is the presence of some extra bodies besides the nuclei. They are found to be present from the megaspore-mother cell stage to the mature embryo-sac stage. They do not stain deeply with hæmatoxylin, but remain pale. The shape of these bodies is varying (Figs. 36–46). Fig. 36 shows the presence of a single such body in a megaspore-mother cell and Fig. 37 of 3 such bodies in an enlarged megaspore-mother cell. Sometimes these bodies have been found to be surrounded by a vacuole (Fig. 36). Fig. 38 illustrates a case of a megaspore-mother cell in which its nucleus and the body in question occupy the micropylar and chalazal poles with a large central vacuole separating them. Further this body persists through the tetrad and other stages of the female gametophyte (Figs. 40–46). The origin and nature of these bodies could not be determined. Karsten (1891) also figures similar bodies. He described them as oily-looking.

Although no such bodies are found in the embryo-sac of *Duabanga sonneratioides*, certain small extra-nuclear karyotin bodies staining jet black with hæmatoxylin have constantly been found to be present in the megaspore-mother cells and dyad cells (Figs. 20 and 21). These may be or may not be surrounded by a hyaline space. These are most probably extrusions of the nucleolus during the prophasic stages of the heterotypic division.

Fertilisation, Endosperm and Development of Embryo and Seed.

The above-mentioned phases of the life-history have been followed only in *Duabanga sonneratioides*.

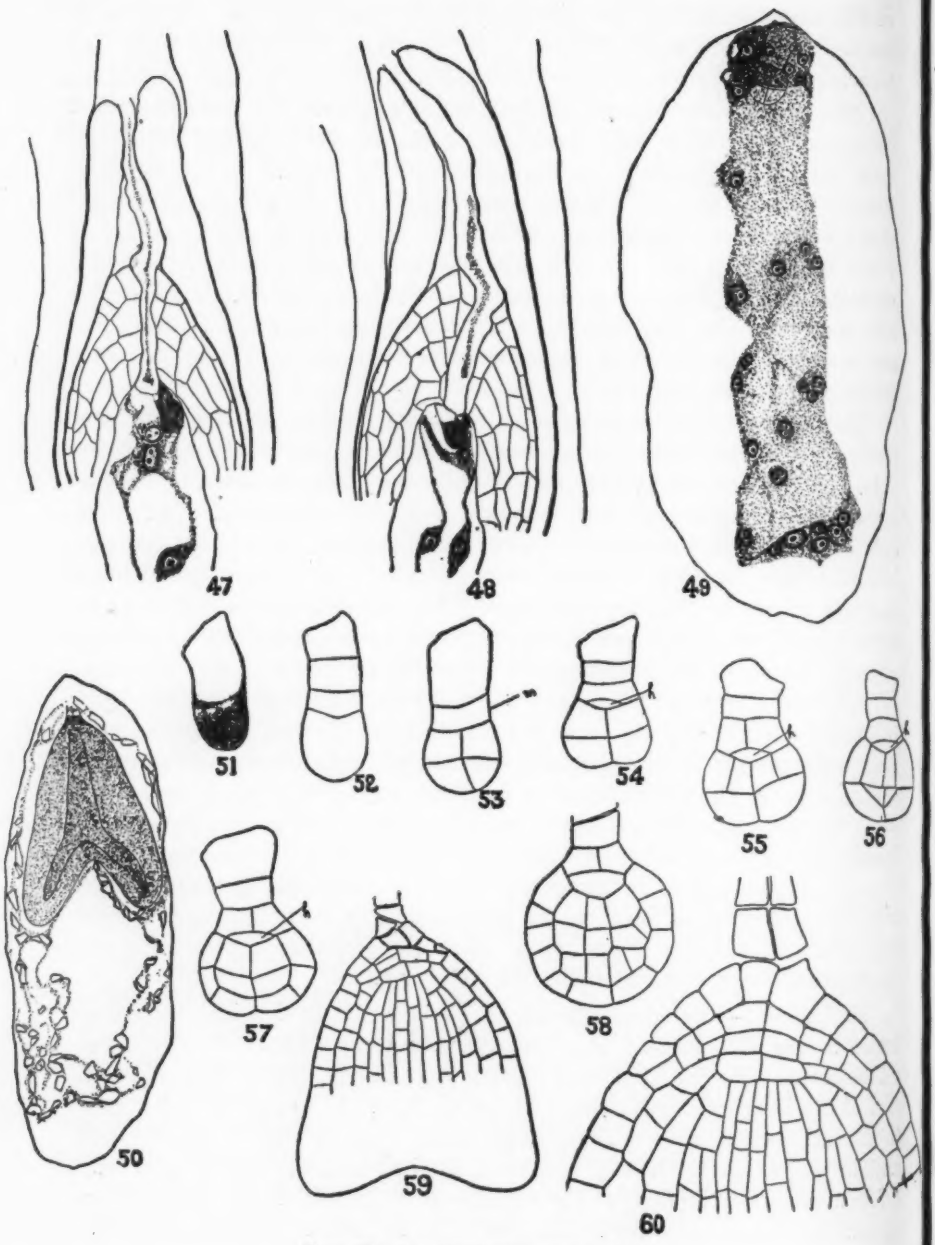
Fertilisation.—Karsten (1891) has made some observations on fertilisation in the embryo-sac of *Sonneratia apetala*, which differ from the results obtained in the present investigation. He found that the embryo-sac, in which fertilisation takes place, bore through the nucellus by a micropylar projection and become situated just below the nucellar epidermis. He further states that the formation of any such aggressive process fails to take place in ovules the embryo-sacs of which are not fertilised. He proceeds

to add that the pollen tube pierces through this process and advances to the egg which remains at the base of the process. This statement has also been incorporated in a recent review of fertilisation by Dahlgren (1927).

The present investigation shows Karsten's observation to be incorrect. Numerous ovules have been examined at the time of fertilisation, but no such process as observed by Karsten could be found. It appears that Karsten had mistaken the broad pollen tube to be the micropylar process of the embryo-sac. He was not able to see any such process in ovules in which fertilisation does not take place because in such cases it is quite natural that no pollen tubes advanced to the embryo-sacs. Some ovules with mature embryo-sacs ready for fertilisation in *Sonneratia apetala* have also been studied, but even a beginning of such a process could not be found in any one of them.

The fertilisation is porogamous. Normal double fertilisation takes place. The pollen tube is very broad and enters by way of the long micropyle. On reaching the apical end of the embryo-sac it proceeds towards one of the synergids and never passes directly to the egg-cell. The broad end of the pollen tube presses upon the synergid and discharges its contents into it. The attacked synergid becomes filled and swollen with dark contents (Figs. 47 and 48) and appears like a structureless mass though retaining the outer form, while the unattacked synergid remains intact. It appears that the male nuclei always pass through the attacked synergid. The synergid breaks at its chalazal end and the contents flow over the eggs. Ultimately one of the male nuclei is found to reach the egg nucleus and the other male nucleus reaches the secondary nucleus. The fusion of the sperm nucleus and the egg nucleus is very slow and for a long time they remain distinct in the egg. On the other hand, the fusion between the second male nucleus and the secondary nucleus is completed earlier. The unattacked synergid degenerates and disappears, while the dark-looking, structureless, attacked synergid is seen to be present until the endosperm is formed (Fig. 48). In this are found often X-bodies probably representing the fragmenting synergid nucleus and the tube nucleus.

Endosperm.—The development of the endosperm is similar to that found in the Lythraceæ. The primary endosperm nucleus divides even before fusion between the sperm nucleus and the egg nucleus is completed. One of the daughter nuclei passes to the chalazal end, while the other goes to the micropylar end. They divide mitotically forming a number of nuclei. These remain mostly restricted to their respective poles and only a few migrate to the sides of the embryo-sac. Cytoplasm also accumulates at these poles just as in the Lythraceæ. On the sides it remains very thin.



FIGS. 47–60. *Duabanga sonneratioides.*

Figs. 47 & 48, upper parts of longitudinal sections of two ovules showing stages in fertilisation and early stages of the development of the endosperm ; Fig. 49, nuclear endosperm with the polar accumulations ; Fig. 50, longitudinal section of an embryo-sac showing cellular endosperm and an advanced embryo ; Figs. 51–60, longitudinal sections of various stages in the development of the embryo ; *h*, hypophysis ; *m*, mother-cell of the hypophysis. Figs. 47, 48 & 59, × 300 ; Fig. 50 × 133 ; Fig. 51, × 600 ; Figs. 49, 52–58 & 60, × 533.

The micropylar accumulation is a little more pronounced than the chalazal one and contains a larger number of nuclei (Fig. 49).

The endosperm is nuclear in early stages. During the advanced stages of the embryo-development, it becomes cellular (Fig. 50). The central cavity of the embryo-sac never gets filled up with endosperm.

Embryo.—The development of the embryo is also found to agree with that of the Lythraceæ. The first division in the oospore is transverse and takes place after a number of endosperm nuclei are formed. This division is followed by one or two more transverse divisions. The pro-embryo thus becomes 3–4-celled.

The terminal cell forms the embryo proper. It divides by two longitudinal divisions giving rise to the quadrants. A transverse division in each of the quadrants forms the octants (Fig. 53). Just as in the Lythraceæ, the four superior octants later form the cotyledons and the stem tip, while the four inferior octants produce the hypocotyl and the primary root except its apex. The hypophysis is formed by the superior daughter-cell of the penultimate cell of the pro-embryo. It differentiates very early, just as the octants are formed. The hypophysis cell is lenticular and separated from its sister cell by a curved wall. In its form and early development, along with the Lythraceæ, it resembles the Onagraceæ (Souèges, 1920). The difference between the hypophysis in the Onagraceæ and these plants is that in the Onagraceæ the hypophysis is formed by the penultimate cell of the pro-embryo directly, while here it is formed by its superior daughter-cell only.

The differentiation of the three histogenic layers proceeds as in the Lythraceæ. The dermatogen differentiates earlier in the inferior octants by periclinal divisions (Fig. 55). The differentiation of plerome also takes place much earlier in the hypocotyl region. The stem tip is formed very late. The hypophysis cell first divides transversely giving rise to two superposed daughter-cells, which later divide and form a plate of cells. The superior cells of this plate form the periblem of the root apex and the inferior cells form the dermatogen and the root cap.

The suspensor is formed by 1 or 2 cells of the pro-embryo not taking part in the development of the embryo proper and the sister cell of the hypophysis. These cells do not seem to undergo any transverse divisions. Thus, the suspensor remains short. The suspensor cells, however, divide longitudinally, the sister cell of the hypophysis dividing first and the rest following it. This form of the suspensor (Fig. 60) agrees with that of species of *Ammania* belonging to the section *Rotala* (Joshi and Venkateswarlu, 1936), and *Cuphea* (Mauritzon, 1934).

Structure of the mature seed.—The mature seed of *Duabanga sonneratioides* is a minute filiform structure measuring about 4 m.m. in length. It is prominently tailed at both the ends, each tail measuring about one-third of the whole length of the seed. The endosperm and the nucellus are destroyed completely by the growing embryo. The mature seed, therefore, is exalbuminous.

The structure of the seed-coat is different in the micropylar region from that of the rest of the body. At the sides, the cells of the outer layer of the outer integument become greatly elongated and a very darkly staining stubstance, probably tannin, is densely deposited in them. The cells of the inner layer of the outer integument divide irregularly and contain some crystals. The cells of the outer layer of the inner integument remain small in size and their walls are thickened, while the cells of the inner layer increase a little in size and a certain substance is deposited in their cavities (Fig. 14).

In the micropylar region, the cells of the outer integument do not change very much, while the inner integument shows some very interesting changes. The cells of the inner layer of this integument remain narrow and elongated. A darkly staining substance, propably of the nature of tannin is deposited in them. The cells of the outer layer increase in size and develop spiral and reticulate thickenings on their walls (Fig. 13*b*). These thickenings are always developed in all mature seeds of this plant. They resemble somewhat similar thickenings developed in the endothecium of the stamens in angiosperms. Similar structure of the micropyle has not been previously recorded in any other angiospermous plant nor such thickenings have been noticed in the cells of the inner integument in any region. Such development, however, has been seen in the cells of the outer integuments, arils, caruncles and seed hairs of several plants such as *Luminitzera racemosa* (Karsten, 1891), *Acorus* (Netolitzky, 1926), etc.

Conclusion.

From the above account it can be at once seen that the embryological features of the family Sonneratiaceæ agree even in details with those of the

Lythraceæ. The structure and development of the anther, the form and structure of mature pollen, the primary archesporium in the ovule, the development of the megaspore-mother cells (even the occasional development of more than one megaspore-mother cell), the structure and development of the nucellus, the formation of parietal tissue, the tetrads, the structure and development of the embryo-sac, particularly the structure of the egg-apparatus, the behaviour of polar nuclei and the early degeneration of the antipodals, the structure and formation of the endosperm (nuclear in the early stages, cellular in later stages and the formation of polar accumulations), the development of the embryo, particularly the differentiation of the hypophysis, and lastly the structure of the seed and testa at the sides show very striking similarities. On embryological grounds there is nothing to separate these genera from the Lythraceæ.

Summary.

The plants studied are *Duabanga sonneratioides* Ham. and *Sonneratia apetala* Lamk.

The primary archesporium in an anther-lobe consists of a hypodermal row of about 10 cells in *Sonneratia* and 20–25 cells in *Duabanga*. The anther-wall outside the tapetum is 4–5 cells thick. The tapetum is of the secretion type. The mature pollen is two-nucleate. The exine bears 3 germ pores arranged in an equatorial manner. Starch is abundantly seen in the mature pollen grains of *Sonneratia apetala*.

The semi-inferior ovary is 5–6-celled in *Sonneratia apetala* and up to 10-celled in *Duabanga sonneratioides*. The ovules are numerous and borne on large axile placentas. They are anatropous, ascending, two-integumented and possess a moderate amount of nucellus. Both the integuments form the micropyle. The parietal tissue above the embryo-sac is 4–5 cells thick. There is a chalazal strand in the nucellus of *Duabanga sonneratioides*. The nucellus and consequently the ovule in the chalazal part in *Sonneratia apetala* shows a marked bend towards the side of the raphe. The mature ovule of *Duabanga sonneratioides* is prominently tailed at both of its ends.

The primary archesporium generally extends to more than a single cell, but usually only one of them develops further. Occasionally two megaspore-mother cells have been found to develop simultaneously. A linear or T-shaped tetrad is formed. The chalazal megaspore forms the embryo-sac. The development of the embryo-sac is normal. The antipodals degenerate early. The form and structure of the egg-apparatus and the behaviour of the polar nuclei corresponds to that of the Lythraceæ. A peculiar pale staining body is present in the megaspore-mother cell and persists even up to

the embryo-sac stage in *Sonneratia apetala*. The fertilisation is porogamous. Double fertilisation and triple fusion take place. No aggressive micropylar process of the embryo-sac as mentioned by Karsten is formed in either of the species studied.

Endosperm is formed before the fusion of the male and the egg nuclei is complete. It is at first nuclear. Later it becomes cellular, but the central part of the embryo-sac is never filled up with cells. In the nuclear stage, the endosperm forms prominent polar accumulations.

The development of the embryo is according to the *Capsella*-type and agrees with that of the Lythraceæ in details.

The seed is exalbuminous. In the seed-coat of *Duabanga*, the cells of the inner integument in the region of the micropyle develop spiral and reticulate thickenings. Such a development has not been previously recorded for any other angiospermous plant.

On the whole the embryological features of the Sonneratiaceæ show no differences from those of the Lythraceæ.

In conclusion, the writer wishes to express his sincere thanks to Professor A. C. Joshi for his kind interest and helpful suggestions during the progress of the investigation. He is indebted to Mr. I. Banerji of Calcutta University for a part of the material of *Duabanga sonneratioides*. He is also thankful to Dr. F. Netolitzky of Cernauti, Roumänia, for some information about the structure of the seed-coat provided in a letter to Prof. A. C. Joshi.

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CONDUCTOMETRIC METHOD FOR THE ESTIMATION OF MICRO-QUANTITIES OF ARGININE.

By Venkatarama Ranganathan.

(From the Department of Biochemistry, Indian Institute of Science, Bangalore.)

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(Communicated by Mr. B. N. Sastri, M.Sc., A.I.C., A.I.I.Sc.)

In a previous communication[8] attention was drawn to the possibility of employing the conductometric method for the estimation of urea in physiological fluids. The present study relates to the extension of the method for the estimation of micro quantities of arginine in physiological fluids and proteins.

Among the methods available for the estimation of arginine mention may be made of the gravimetric method of Kossel and Staudt,[5] the alkali distillation method due to van Slyke,[10] and the arginase method of Jansen.[4] The last method as improved by Hunter and Dauphinee[2] is the one of choice in view of the fact that the determination involves the use of the specific enzyme arginase. The van Slyke procedure does not always give reliable values, as for instance, when applied to its determination in the non-protein nitrogenous fraction of various plant materials (Bhagvat and Sreenivasaya[1]). The method also gives erroneous values when applied to the determination of arginine in the basic fraction in the usual van Slyke procedure for proteins (Plimmer[7]). Kossel's method is time consuming since it involves the isolation of amino acid and is liable to errors. The colorimetric method of Weber[12] is rather unreliable when applied to protein hydrolysates and physiological fluids owing to the presence of interfering substances. Very recently the manometric method of Krebs[6] has been applied by Hunter and Pettigrew[3] for the determination of arginine in proteins, making use of the manometric gas analysis apparatus of van Slyke and Neil. The conductometric method, which can be applied for the determination of micro-quantities of arginine, possesses distinct advantages over other methods, with regard to rapidity, quantity of research material and accuracy.

Experimental.

Arginase.—The press juice obtained from a fresh ram's liver was diluted ten-fold and after centrifuging the clear liquid was concentrated by freezing (Sastri[9]), to about half the volume of the original press juice. The extract prepared in this way was found to be very active and retained its activity on storage in the ice chest for several weeks. The extract was dialysed in collodion bags before use.

Urease.—The soya enzyme used in this investigation was prepared according to the procedure of van Slyke and Cullen.[11] A dialysed 10 per cent. extract of the preparation was employed.

A mixture of enzymes arginase and urease in the ratio of 2 : 1 by volume was used throughout. Such a mixture was found to retain its activity on storage in ice chest for several weeks. 2 ml. of the enzyme mixture was incubated in a conductivity cell for three hours with 4 ml. of aspergine and glycine, to test its deamidase and deaminase activities respectively. The changes in conductivity brought about were negligibly small and it may therefore be safely assumed that the enzyme reagent was free from determinable quantities of deamidase and deaminase.

Determination of Conductivity.—The arrangement described in the previous communication,[8] was used. 1 ml. of arginine solution was placed in a conductivity cell together with 0·5 to 2 ml. of M/15 phosphate buffer of pH 7, and enough conductivity water added to make the volume 4 ml. The contents of the cell were well mixed and the cell immersed in a thermostat maintained at 30·0°C., ± 0·1°C. After about half an hour when the resistance attained a steady value, 2 ml. of a dialysed urease-arginase mixture raised to the same temperature as that of the thermostat was added, the time of addition of the enzyme being noted. Readings were taken at intervals of every minute in the initial stages, and during the later stages, at intervals of 5 minutes. An excess of urease was generally employed to prevent any accumulation of urea and the quantity of enzyme mixture was such, that the whole amount of arginine was hydrolysed in about 30 minutes. Table I gives the results obtained with 2·99 mg. and 0·15 mg. of arginine.

TABLE I.

Time in mins.	2·99 mg.		0·15 mg.	
	Balancing resistance in Ohms	Bridge reading	Balancing resistance in ohms	Bridge Reading
	Before adding enzyme			
0	111	575	510	690
10	,,	550	,,	675
15	,,	545	,,	671
20	,,	544	,,	670
	After adding enzyme			
1	190	565	630	589
2	,,	536	,,	579
3	,,	504	,,	568
4	,,	471	,,	558
5	,,	440	,,	546
8	,,	340	,,	520
11	,,	265	,,	510
15	,,	205	,,	500
20	,,	162	,,	485
28	,,	153	,,	480
31	,,	155	,,	481
46	,,	154	,,	480

The readings were plotted on a graph, with the time on the X-axis and the resistance on the Y-axis. The resistance at 0 time can be ascertained by extrapolation. The total change in resistance gives a measure of the electrolyte released during the reaction, and hence the amount of arginine.

Various amounts of arginine were taken and the conductivity changes accompanying the hydrolysis were measured. The results are given in Table II.

TABLE II.

Relation between conductivity and quatnity of arginine.

Quantity of arginine in mg.	2·99	2·39	1·79	1·49	1·21	0·60	0·30	0·15
Conductivity × 10^4	5·80	4·69	3·56	3·05	2·52	2·31	0·75	0·45

It may be mentioned that the change in conductivity is essentially due to the hydrolysis of urea released from arginine. The change in conductivity can be referred to the graph (Fig. 1) for obtaining arginine values.

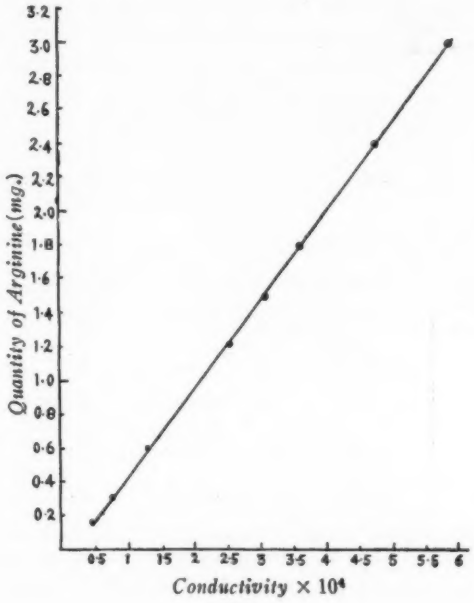

FIG. 1.

Estimation of arginine in proteins.—1 g. of the protein was weighed out into a round-bottomed flask and hydrolysed with 20 ml. of 25 per cent. sulphuric acid for 24 hours at 110°C. The acid was quantitatively removed by baryta and the precipitate extracted thrice with boiling water to ensure the complete removal of all nitrogenous matter from the precipitate. The combined filtrates were concentrated to about 5 ml. in a 500 ml. Claisen's flask under reduced pressure at a temperature not exceeding 50°C. The residue was quantitatively transferred to a measuring flask and made up to 25 ml. 1 ml. of solution was found to be sufficient for each determination the quantities of both enzyme and buffer used being 2·0 ml.

To test the applicability of the method for the estimation of arginine, in proteins, two set of experiments were conducted :—

I. Known quantities of arginine were added to a definite volume of the various protein hydrolysates—1 ml.—and the quantity of added arginine estimated by the conductometric procedure; and

II. The values obtained for various proteins, by the conductometric method were compared with those obtained by the Hunter and Dauphinee's method.[2] The results obtained with additions of known quantities of arginine are given in Table III, after making allowance for the arginine that is already present in the protein hydrolysates.

TABLE III.

Estimation of added arginine.

Quantity of arginine added mg.	mg. of arginine estimated after addition to the hydrolysates of			
	Casein	Gelatin	Fibrin	Edestin
2·82	2·79	2·75	2·78	2·80
2·11	2·05	2·04	2·09	2·10
1·41	1·04	1·04	1·42	1·36
0·70	0·71	0·96	0·71	0·70

The results obtained by the conductometric method and the Hunter and Dauphinee's method are compared in Table IV.

TABLE IV.

Comparison of the arginine content by the conductometric and Hunter and Dauphinee's procedure.

Proteins	Casein	Gelatin	Edestin	Fibrin	Hæmo-globin	Witte's Peptone
Conductometric method in mg. ..	1·36	2·83	5·30	2·10	1·49	2·51
Hunter and Dauphinee's method in mg. ..	1·40	2·84	5·38	2·23	1·50	2·40

The close agreement between the two sets of values definitely shows that the conductometric method can be applied for the estimation of arginine in proteins.

Procedure for estimation in micro-quantities of proteins.—The quantity of protein necessary for the estimation of arginine is only 50 mg. A weighed quantity of the material was hydrolysed in the usual way in a 5 ml. hard glass test-tube fitted with a long air condenser with 1 ml. of 20 per cent. sulphuric acid. The digest was transferred to a beaker and the sulphuric acid was quantitatively removed by adding baryta. After repeatedly washing the precipitate, the filtrate was concentrated to a volume of 2·5 ml. as before. 0·5 to 1·0 ml. was taken for each determination according to the nature of the protein employed. The results obtained for casein and gelatin are tabulated below, the volume of the hydrolysates used in each experiment being 1 ml.

TABLE V.

Protein	Weight taken mg.	Arginine content by conductometric method in mg.	Arginine content by Hunter and Dauphinee's macro-method in mg.
Gelatin I ..	75·0	2·0	2·10
Gelatin II ..	49·0	1·37	1·39
Casein ..	53·7	0·75	0·76

The quantity of material taken for the estimation by the Hunter and Dauphinee's procedure was about 1 g. The values obtained by the two methods agree within experimental errors.

It will be seen that the conductometric method while it claims equality with Hunter and Dauphinee's procedure with respect to accuracy (1 per cent.) possesses the merit of requiring smaller quantities of research material (50 mg. of protein). Again, the estimation is complete in an hour, while the previous methods require as many as 24 hours. By using appropriate cells, it should be possible to estimate still smaller quantities of arginine; the total volume of liquid used in these experiments was 6 ml., and the amount actually required for dipping the electrodes in the liquid was 2 ml. Hence by using only 2 ml. it will be possible to estimate 0·05 mg. of arginine with the same degree of accuracy. The procedure is simple and does not require the use of standard solution. When the presence of urea is suspected, a control urea determination will be necessary.

Summary.

A micro-method for the estimation of arginine based on the measurement of the change in conductivity resulting from the hydrolysis of arginine

by the use of a mixture of arginase and urease, has been described. The method gives reliable values within 1 per cent. error for quantities as low as $0{\cdot}5 \times 10^{-4}$ g. of arginine.

The method has been applied for the estimation of arginine in proteins and the values obtained compared with other methods. The time taken for one estimation is less than an hour, and the quantity of proteins required is only 50 mg.

My thanks are due to Mr. B. N. Sastri for many helpful suggestions and also to Prof. V. Subrahmanyan, for his keen interest in the work. My thanks are also due to the Madras Government for the award of a Research Scholarship.

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Corrigenda.

Volume V, No. 4, Page 168.

Explanation of Plates.

Omit Fig. 4 and its explanation.
Read Figs. 5, 6 and 7 *as* Figs. 4, 5 and 6.

Fig. 7.—Egg of *Lycastis*. Note the double-rimmed batonettes and fusion of chromophilic rims. 15 × 100. Bellows extension 8″.

CHROMOSOME BEHAVIOUR IN *TRITICUM* HYBRIDS AND ALLIED GENERA.

I. Interspecific Hybrids with *Triticum Timopheevi.*

By Dontcho Kostoff.

(From the Institute of Genetics, Academy of Sciences of USSR, Moscow.)

Received January 19, 1937.

(Communicated by Prof. C. R. Narayan Rao, M.A.)

The studies of Kihara and Lilienfeld (1934) showed that in the hybrids *Tr. ægilopoides* ($n = 7$) × *Tr. Timopheevi* ($n = 14$), *Tr. Timopheevi* × *Tr. pyramidale* ($n = 14$), *Tr. Timopheevi* × *Tr. dicoccum* ($n \times 14$), and *Tr. Timopheevi* × *Tr. persicum* ($n = 14$) genom A (7 chromosomes) of $n = 7$ -chromosome group (*ægilopoides*) and of $n = 14$ -chromosome group conjugate with one genom of *Tr. Timopheevi*, while the other genom of *Tr. Timopheevi* is not homologous with genom B of *durum* group. This they called genom G. Hence *Tr. Timopheevi* has the following genom formula AG in contrast to the other *Triticum* species of *durum* group that have the formula AB. They did not succeed in crossing *Tr. Timopheevi* with *Tr. vulgare* group and did not have direct evidence of the degree of the homology of genom G in respect to the third genom of the genus *Triticum*, namely, genom C. (For this genom they use the designation D.) They found, however, that *Timopheevi* genoms are not homologous with C, D, E, F, T and some others *ægilops* genoms.

We were more successful in this respect. We grew hybrids from the following cross combinations :

Hybrids obtained	Somatic chromosome number
Triticum Timopheevi × *Tr. monococcum* var. *Hornemani* ..	21
Tr. Timopheevi × *Tr. mon.* var. *flavescens*	21
Tr. Timopheevi × *Tr. mon.* var. *pseudovulgare*	21
Tr. monoc. var. *flavescens* × *Tr. Timopheevi*	21
Tr. Timopheevi × *Secale cereale*	21
Tr. Timopheevi × *Haynaldia villosa*	21
Tr. Timopheevi × *Tr. persicum* var. *stramin*	28

Hybrids obtained	Somatic chromosome number
Tr. persicum str. × *Tr. Timopheevi*	28
Tr. Timopheevi × *Tr. Spelta*	35
Tr. Spelta × *Tr. Timopheevi*	35
Tr. Timopheevi × *Tr. vulgare* var. *turcicum*	35
Tr. vulgare turcicum × *Tr. Timopheevi*	35
Tr. vulgare rigidum × *Tr. Timopheevi*	35
Tr. compactum × *Tr. Timopheevi*	35
Tr. Timopheevi × *Tr. vulgare Khoros.*	35
Tr. vulg. Khorosanicum × *Tr. Timopheevi*	35
Tr. vulg. Novinka × *Tr. Timopheevi*	35
Tr. vulg. ferrugin. × *Tr. Timopheevi*	35
Tr. Macha × *Tr. Timopheevi*	not determined
Tr. durum × *Tr. Timopheevi*	28
Tr. polonicum × *Tr. Timopheevi*	28
Tr. sphærococcum × *Tr. Timopheevi*	35
(*Tr. compactum* × *Tr. Timopheevi*) × *Tr. monococcum pseudovulgare*	22
(*Tr. vulgare* × *Tr. monococcum*) × *Tr. Timopheevi*	28, 29
(*Tr. compactum* × *Tr. Timopheevi*) × *Tr. vulgare*	not determined
(*Tr. persicum* × *Tr. Timopheevi*) × *Tr. turgidum*	28
(*Tr. persicum* × *Tr. Timopheevi*) × *Tr. monococcum* ..	21
(*Tr. persicum* × *Tr. Timopheevi*) × *Tr. monococcum* ..	21
(*Tr. persicum* × *Tr. Timopheevi*) × *Tr. monococcum* ..	22
(*Tr. persicum* × *Tr. Timopheevi*) × *Secale cereale*	21
(*Tr. Spelta* × *Tr. Timopheevi*) × *Tr. monococcum*	22
(*Tr. Spelta* × *Tr. Timopheevi*) × *Tr. Spelta*	39
(*Tr. Timopheevi* × *Tr. monococcum*) × *Tr. turgidum* type ..	42

In studying the reduction division in the hybrids *Tr. Timopheevi* × *Tr. monococcum* (and the reciprocal) we found that the chromosomes of *Tr. monococcum* do conjugate with seven of *Tr. Timopheevi*. We studied the chromosome association in 9 plants.

The following data were obtained from a more detailed study of the meiosis in *Tr. Timopheevi* × *Tr. monococcum* var. *flavescens*:

I Metaphase in Triticum Timopheevi × Tr. monococcum *hybrid.*

PMC's	Quadrivalents	Trivalents	Bivalents	Univalents
9	..	..	7	7
11	..	..	6	9
7	..	..	5	11
6	..	1	6	6
4	..	1	5	8
1	1	..	5	7

In the majority of cases the bivalents and polyvalents in the I metaphase in the PMC are joined with a single terminal or subterminal chiasma. Occasionally one pair of chromosomes had two chiasmata, and very rarely cells with two pairs of chromosomes each with two chiasmata were found.

In studying the hybrid *Tr. Timopheevi* × *Tr. durum* we found most frequently 9 and 10 bivalents, but occasionally 8, 11 and 12 were observed too. In a few cases even 13 bivalents were found. Cells with one trivalent chromosome were often noted, while in several PMC even one quadrivalent (in one case possibly pentavalent) chromosome was found. The majority of the bivalents had a single subterminal or terminal chiasma. In the majority of cells, however, two bivalents had each two chiasmata. We are not sure whether two particular pairs always form each two chiasmata, since they could not be identified. Trivalents and polyvalents were occasionally seen too.

The hybrid *Tr. Timopheevi* × *Tr. persicum* seems to form many more bivalents than *Tr. Timopheevi* × *Tr. durum*. Most frequently 11, 12 and 13 bivalents were observed. In exceptional cases even 14 were seen, but 9 and 10 occurred occasionally too. Trivalents and quadrivalents were also found in some of the PMC. This hybrid had quite often PMC in which three of the bivalents had two chiasmata. Kihara and Lilienfeld (1934) found that in this hybrid " Es kommen 9-14 Bundungen vor, am haufigsten 12 ".

If we take into consideration the fact that all the *monococcum* chromosomes usually conjugate with 7 chromosomes of *Tr. Timopheevi*, we may assume that *monococcum* genom (A) is homologous (though not quite) with one genom (7 chromosomes) of *Tr. Timopheevi*, while the other genom of *Tr. Timopheevi* is partially homologous with genom B of *Tr. persicum* as

the data of Kihara and Lilienfeld as well as our own data show. We cannot quite agree with Kihara and Lilienfeld in considering the second genom of *Tr. Timopheevi* as a quite different one from genom B, because in some cases even all its 7 chromosomes conjugate with those of genom B. On the other hand, the second genom of *Tr. Timopheevi* although closely related with B genom is more differentiated than the first genom in respect to genom A. All these facts do not allow us to separate totally the second genom of *Tr. Timopheevi* from genom B as Kihara and Lilienfeld did and designated it with G, therefore we are calling it genom β. Genom β is partially homologous with genom B. Hence *Tr. Timopheevi* really has the following genom formula : AA $\beta\beta$.

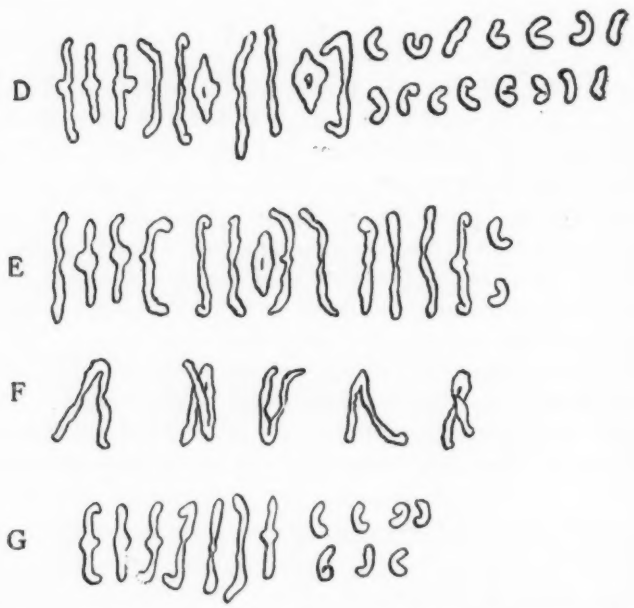

FIG. 4.—Chromosome dissociation during the first metaphase in *Triticum Timopheevi* hybrids:-

D—The chromosomes from a pollen mother cell of *Tr. compactum* ($n = 21$) × *Tr. Timopheevi* hybrid, 10 bivalents and 15 univalents.

E—The chromosomes from a pollen mother cell during the first meiosis of *Tr. Timopheevi* ($n = 14$) × *Tr. persicum* ($n = 14$) hybrid, 13 bivalents and 2 univalents.

F—Polyvalent chromosomes from various pollen mother cells of the hybrid *Tr. Timopheevi* × *Tr. persicum*.

G—The chromosomes from a pollen mother cell during the first meiosis of the hybrid *Tr. Timopheevi* ($n = 14$) × *Tr. monococcum* ($n = 7$), 7 bivalents and 7 univalents.

Further studies were directed toward the investigation of the chromosome behaviour of genom β (that had low affinity with their corresponding chromosomes of genom B) in respect to the chromosomes of C genom in the hybirds produced between *Tr. Timopheevi* and the wheat of *vulgare* group. Therefore the chromosome behaviour in the I metaphase of the PMC was studied in the hybrids *Tr. vulgare* × *Tr. Timopheevi*, *Tr. compactum* × *Tr. Timopheevi*, *Tr. sphærococcum* × *Tr. Timopheevi* and finally *Tr. Spelta* × *Tr. Timopheevi*.

In doing this we found that in the hybrids *Tr. vulgare* var. *rigidum* × *Tr. Timopheevi* most frequently 9-12 bivalents were found with terminal or subterminal chiasmata, similar to those of *Tr. Timopheevi* × *Tr. durum* and *Tr. Timopheevi* × *Tr. persicum*. Trivalents (1 and rarely 2) were occasionally found to occur. In a few cases even polyvalents were seen. Two and sometimes three of the bivalents had each two chiasmata.

The hybrids *Tr. compactum* × *Tr. Timopheevi* and *Tr. Spelta* × *Tr. Trimopheevi* behave in almost the same way. Both had most frequently 9-12 bivalents, while the hybrid *Tr. sphærococcum* × *Tr. Timopheevi* had somewhat less bivalents. In this hybrid we found most frequently 8-10 bivalents, but 7, 11 and 12 were also seen, as well as polyvalents and trivalents.

The above observations show that the chromosomes of β genom do not seem to have an affinity in respect to the chromosomes of genom C of *vulgare* group.

The number of bivalents formed in the hybrids *vulgare* × *Timopheevi*, *Spelta* × *Timopheevi* and *compactum* × *Timopheevi* remind us of those in the hybrid *Timopheevi* × *persicum*, while those formed in *Tr. sphærococcum* × *Tr. Timopheevi* remind us of those formed in *Tr. Timopheevi* × *Tr. durum*.

The hybrid *Tr. Timopheevi* × *Haynaldia villosa* had none, one, or a few bivalents. It had 0·81 chiasmata per cell and 0·04 chiasmata per chromosome. Consequently genoms A and β of *Tr. Timopheevi* are not homologous with genom V of *Haynaldia villosa*.

Previous investigations have shown that genom S of *Secale cereale* is not homologous with genoms A, B and C of *Triticum*. In 1935 we grew two hybrids *Triticum Timopheevi* × *Secale cereale* and studied the relation between the genoms A and β of *Tr. Timopheevi* and genom S of *Secale cereale* ($n = 7$). One of the hybrids was possible to study cytologically, because the anthers of the other degenerated at a very early stage of development. We found usually 21 univalent chromosomes or 19 univalents and one bivalent during the first meiotic metaphase. The bivalent had usually one terminal

chiasma. In only a few instances two bivalents with terminal chiasmata were observed. In only one pollen mother cell a trivalent group with terminal chiasmata was found. These observations indicate that the chromosomes of A and β genoms of *Tr. Timopheevi* are not homologous with the chromosomes of S genom of *Secale cereale*. In other words, genoms A and β are not homologous with genom S.

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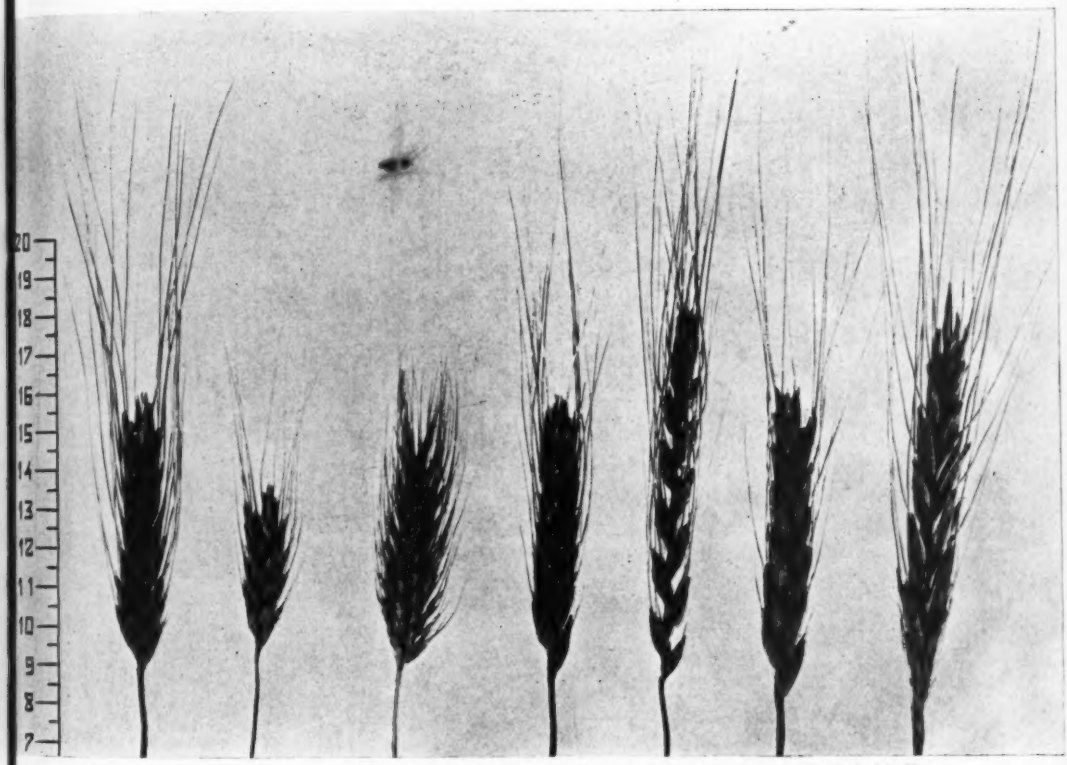

FIG. 1.—(*From left to the right*): Spikes of (1) *Triticum Timopheevi*; (2) hybrid *Tr. Timopheevi* × *Haynaldia villosa*; (3) *Haynaldia villosa*; (4) hybrid *Tr. persicum* × *Tr. Timopheevi*; (5) *Tr. persicum*; (6) hybrid *Tr. Timopheevi* × *Tr. polonicum*; (7) *Tr. polonicum*.

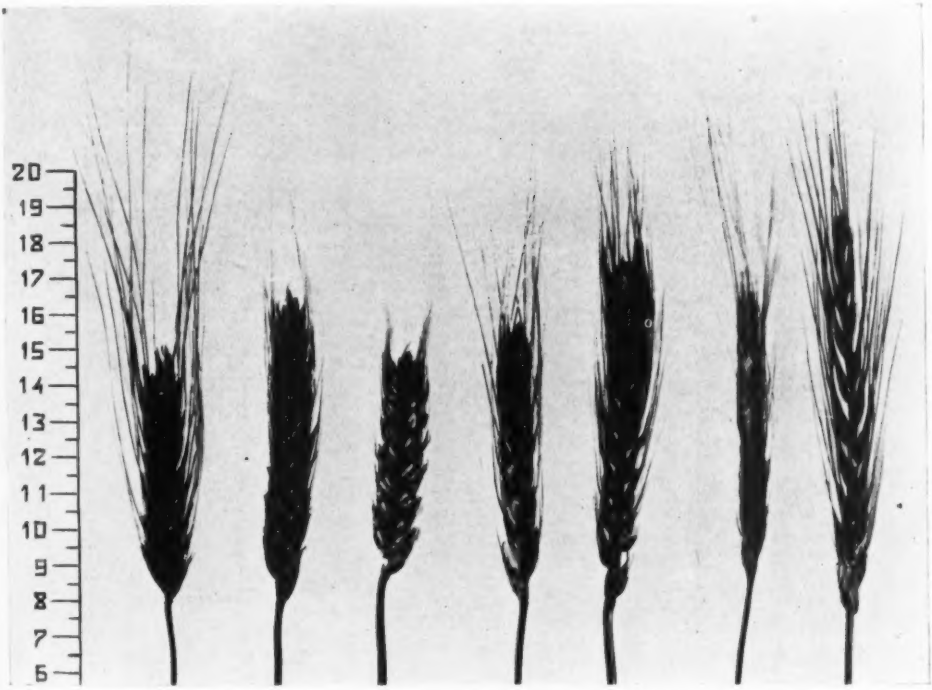

FIG. 2.—(*From left to the right*): Spikes of (1) *Tr. Timopheevi*; (2) hybrid *Tr. compactum* × *Tr. Timopheevi*; (3) *Tr. compactum*; (4) hybrid *Tr. vulgare* var. *ferrugineum* × *Tr. Timopheevi*; (5) *Tr. vulgare* var. *ferrugineum*; (6) hybrid *Tr. vulgare* var. *rigidum* × *Tr. Timopheevi*; (7) *Tr. vulgare* var. *rigidum*.

FIG. 3.—(*From left to the right*): Spikes of (1) *Tr. Timopheevi*; (2) hybrid *Tr. Timopheevi* × *Secale cereale*; (3) *Secale cereale*; (4) *Tr. Spelta* (with awns); (5) hybrid *Tr. Spelta* (with awns) × *Tr. Timopheevi*; (6) hybrid *Tr. Spelta* (awnless) × *Tr. Timopheevi*; (7) *Tr. Spelta* (awnless).

STUDIES ON POLYPLOID PLANTS.

XVI. *Nicotiana rustica* × *Nicotiana tabacum* Amphidiploid.

By Dontcho Kostoff.

(*From the Institute of Genetics, Academy of Sciences of USSR, Moscow.*)

Received January 19, 1937.

(Communicated by Prof. C. R. Narayan Rao, M.A.)

In centrifuging germinated seeds from various plants, we produced different types of chromosomal chimeras, including such with a complete chromosome duplication (Kostoff, 1935 and unpublished). Rajably (in press), one of our students, produced such chimeras in various plants and even a tetraploid *Matthiola*. We applied this method for inducing chromosome doubling in hybrid seedlings in order to produce fertile amphidiploid plants from sterile hybrids. We produced first a large amount of seeds in crossing *N. rustica* (a *texana* type) with *N. tabacum* var. *Basma*. The seeds were sown in filter paper. They germinated during a very long period of time. We used only those that germinated during the first 12 days. They were centrifuged when just started to germinate in series, each series being centrifuged several days, and then the seedlings were planted in pots (with soil + sand). When the seedlings grew about 1½ cm. high they were transplanted each in a pot.

Normal *N. rustica* and *N. tabacum* hybrids are self-sterile. If a chromosome doubling has occurred following centrifuging in a cell and a plant or a branch have developed from such a cell such a hydrid plant or branch, having a double chromosome number, should be fertile, or highly fertile being amphidiploid. We raised 81 centrifuged hybrids. One of them was chromosomal chimera, having sterile top and one fertile side branch. The fertile branch had a double chromosome number. During the first meiotic metaphase we counted 46, 47, 48, 49 and 50 chromatin bodies ; most frequently, however, 48 were found. During the second metaphase we found usually metaphase plates with 48 chromosomes, though such with more than 48 as well as less than 48 chromosomes were also seen. In a few pollen mother cells the chromosome number in both second metaphase plates was possible to count. The chromosome number in both plates together was 96. Considering the fact that F_1 hybrids have 48 somatic chromosomes (24 *rustica* + 24 *tabacum*), the fertile branch of the sterile hybrid represents an amphidiploid *N. rustica* × *N. tabacum*. It was of interest to study the chromosome number of the

sterile top of the same hybrid that had a fertile branch. Second metaphases of it were studied. The chromosome number in both metaphase plates were counted and they were most frequently 48. In a few instances 49, 50 and 51 were counted. They can be interpreted by assuming division of univalent chromosomes during the first meiosis.

In studying the first meiotic division of the fertile branch we found that occasionally univalent as well as trivalent chromosomes occurred. In a few instances even a quadrivalent was found.

The occurrence of second metaphases with more and less than 48 chromosomes is not difficult to interpret when we consider the fact that univalent chromosomes distribute usually at random and that two chromosomes of a trivalent group usually go to one pole while the third chromosome goes usually to the opposite pole.

Fig. 2.

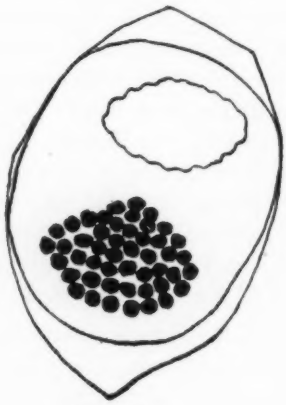

Fig. 3.

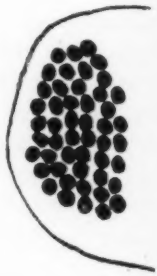

Fig. 4.

Fig. 2.—Polyvalent chromosomes from an amphidiploid plant *N. rustica–tabacum* (PMC).

Figs. 3 and 4.—Second metaphases from the amphidiploid *N. rustica* × *N. tabacum*; Fig. 3 with 48 chromosomes and Fig. 4 with 49 chromosomes.

The members of the polyvalent chromosomes are most probably: one *rustica* + two *tabacum* chromosomes; or two *rustica* + one *tabacum* forming trivalent groups; while two *rustica* + two *tabacum* forming a quadrivalent group. Such polyvalents can be even *a priori* expected since in F_1 hybrids a large number of bivalent chromosomes (16–24) are formed allosyndetically between *N. rustica* and *N. tabacum* chromosomes. The bivalent chromosomes in F_1 hybrids result from allosyndesis of *rustica* chromosomes with those of *tabacum* but not autosyndaticly, because in the haploid *N. rustica* only occasionally one bivalent chromosome is formed autosyndetically and in the haploid

N. tabacum one or two bivalents occasionally occur following autosyndesis (Kostoff, 1936 *a*).

Selfing the fertile branch of *N. rustica* × *N. tabacum* hybrid we produced progeny of : (1) highly fertile plants, (2) partially fertile plants and (3) highly sterile plants. The amphidiploid *rustica-tabacum* is not constant because *N. rustica* chromosomes have segments homologous with segments of *N. tabacum* chromosomes (or whole *N. rustica* chromosomes have homologous chromosomes of *N. tabacum* genom), which lead to formation of trivalents and univalents and of quadrivalents. The univalent and polyvalent chromosomes are responsible for formation of gametes with unequal chromosome number (more than 48 and less than 48). From such gametes chromosomal aberrants originate with reduced fertility. Chromosome reconstruction following crossing-over in the polyvalent groups between *rustica* and *tabacum* chromosomes may be also responsible for the unconstancy of the amphidiploid.

The flowers of the fertile amphidiploid branch and of its fertile progeny were somewhat broader. The plants of the fertile progeny (amphidiploids) were not larger than the F_1 sterile plants. They only had somewhat coarser leaves and stems.

It should be mentioned here that Eghis (1927) and Rybin (1927) reported an amphidiploid *tabacum-rustica* produced by crossing a hybrid having two *tabacum* and one *rustica* genoms with *N. rustica*. Their amphidiploid was according to Sarana (in press) also unconstant. Pratassenya (1935) doubled the somatic chromosome number in *N. rustica* var. *Erbassanta* × *N. tabacum* "Dubek" F_1 hybrids by cuttings and inducing callus regenerations. These amphidiploids are highly fertile.

Chromosome doubling in nature occurs under the influence of various environmental factors.

When we consider the fact that *N. tabacum* species ($n = 24$, $2n = 48$) is an amphidiploid plant of *N. sylvestris* ($n = 12$, $2n = 24$) and *N. tomentosiformis* (Rusbyi) ($n = 12$, $2n = 24$), and that *N. rustica* ($n = 24$, $2n = 48$) is an amphidiploid of *N. paniculata* ($n = 12$, $2n = 24$) and probably *N. undulata* ($n = 12$, $2n = 24$) (v. Kostoff, 1936), or (*N. Raimondii*) the amphidiploid plant *N. rustica* × *N. tabacum* with 96 somatic chromosomes represents an octaploid form combining the genoms of four species each having 24 chromosomes, namely : *N. sylvestris* ($2n = 24$) + *N. tomentosiformis* ($2n = 24$) + *N. paniculata* ($2n = 24$) + *N. undulata* ($2n = 24$).

We are greatly indebted to Z. K. Vishemirskaya and V. F. Botcharova for help throughout this work.

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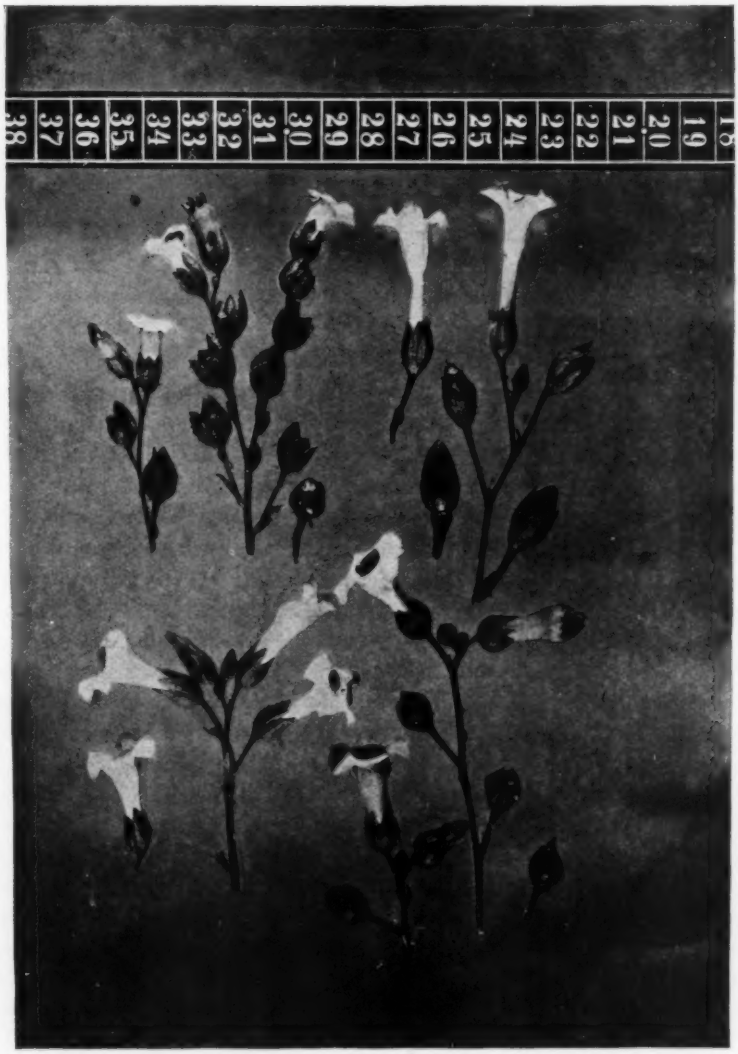

FIG. 1.—*a*—*N. rustica*; *b*–*N. tabacum*; *c*-F_1 (*N. rustica* × *N. tabacum*); *d*—amphidiploid (*N. rustica* × *N. tabacum*).

STUDIES ON POLYPLOID PLANTS.

XVII. *Nicotiana multivalvis* ($2n = 48$) × *Nicotiana suaveolens* ($2n = 32$) Amphidiploid ($2n = 80$).

By Dontcho Kostoff.

(*From the Institute of Genetics, Academy of Sciences of USSR, Moscow.*)

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Hybrids between *N. multivalvis* and *N. suaveolens* can be easily produced in both directions. They are self-sterile and form usually abortive pollen. Only a very insignificant percentage of the pollen stain deep red in aceto-carmine, which indicates that they probably are viable. In autumn, however, when the temperature drops relatively low at night, the hybrids begin to form a somewhat larger amount of viable pollen. It seems that the low temperature influences the procedure of the meiotic processes inducing formation of restitution nuclei, dyads and pollen with somatic chromosome number. The observation on other hybrids (Kostoff, 1933) suggested that high temperature influences the procedure of the meiosis in a similar way and induce a formation of a large amount of viable pollen with somatic chromosome number. Considering these circumstances, we attempted to increase the formation of viable non-reduce gametes under the influence of high temperature. Hybrids of the F_1-generation were put into thermostats with glass walls at a temperature of 40–42°C., 4 days for 3–4 hours each day. The buds and the flowers of the hybrids grown in more shady places of the green house dropped down, while those of the hybrids grown near the opened windows at sunny places were not affected as much. When the small floral buds (in which it was supposed that meiosis occurs at the time of exposure at high temperature) developed into flowers, the plants were selfed and pollinated with *N. Sanderæ* ($n = 9$) having red flowers and then put again in the thermostat for four days, 3–4 hours each day. A large number of the flowers dropped. About 10 capsules did not drop at the time of exposure. Three reached the maturity. Twelve seeds were produced out of these three capsules. Nine of them were shrunken, one was very large but flat and two were round. Only one germinated and a plant developed that was very much like the F_1-hybrids

N. multivalvis × *N. suaveolens.* This plant was self-fertile. It had 40 chromosomes in the first and in each second metaphase.

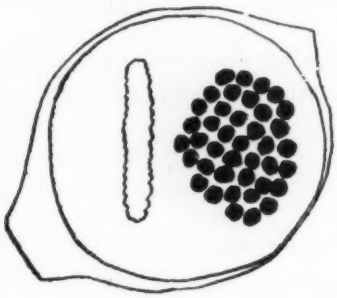

FIG. 1.—Second metaphase of the amphidiploid hybrid *N. multivalvis* × *N. suaveolens* with 40 chromosomes (PMC).

These data show that the plant is an amphidiploid *N. multivalvis* × *N. suaveolens* plant. There are three most probable possibilities about the origins of this plant:

1. A non-reduced egg cell has been fertilized with a sperm having a non-reduced chromosome number. *N. Sanderæ* pollen-tubes have made a path through style of the F_1-hybrid flower, thus facilitating the passing of the *multivalvis-suaveolens* pollen-tubes of pollen grains with somatic chromosome number through the style of the F_1-hybrid.

2. The plant has originated from an egg cell with 80 chromosomes formed following non-occurrence of both meiotic divisions, *N. Sanderæ* pollen-tube has activated parthenogenetic development of this egg cell. Such cases occasionally occur in *Nicotiana* species hybrids. Such a case was reported in *Triticum* too (Kostoff, 1936).

3. The plant has originated from an egg with somatic chromosome number and subsequent somatic doubling under the influence of the high temperature, the parthenogenetic development being induced by the pollen-tube of *N. Sanderæ*.

Amphidiploid *Nicotiana multivalvis* × *N. suaveolens* had much larger cells than either parent and F_1-hybrid *N. multivalvis* × *N. suaveolens.* Stomata cells as well as the pollen grains were measured. We shall give here some data obtained from measuring the pollen grains in the amphidiploid and the parental species.

TABLE I.
Diameter of the pollen grains.

Plants	Ocular micrometer divisions											Total number measured
	13	14	15	16	17	18	19	20	21	22	23	
N. multivalvis	2	5	16	12	3	..	..	..	..	..	..	38
N. suaveolens	..	2	6	24	4	..	..	..	..	..	..	36
Amphidiploid *N. multivalvis* × *N. suaveolens*	..	..	..	..	1	6	13	13	3	1	2	39

The data given in Table I indicate that the diameters of the pollen grains of the parental species are much smaller than those of the amphidiploid hybrid, *N. multivalvis* having an average of 15·2, *N. suaveolens*—15·5, while the amphidiploid hybrid *N. multivalvis* × *N. suaveolens* an average diameter of 19·56 units (divisions of the ocular micrometer).

It should be also pointed out here that *multivalvis suaveolens* amphidiploid sets a large amount of seeds. It set on the average many more seeds (106) per capsule than *N. suaveolens* (90) but less than the maternal species *N. multivalvis* (656). This amphidiploid sets many more seeds than the amphidiploid *N. rustica* × *N. paniculata*, because the latter forms often polyvalent chromosomes and has more irregular meiosis than *N. multivalvis* × *suaveolens* amphidiploid, forming usually bivalents during the first meiosis.

The progeny of the amphidiploid *N. multivalvis* × *N. suaveolens* were uniform and like their parental amphidiploid plant and F_1-plants. The amphidiploids were more robust than F_1-hybrids and the flowers were slightly larger. They often formed flowers with more than five lobes and capsules with more than two carpels—characters that are typical for *N. multivalvis*. They were, however, less pronounced in the amphidiploids than in the maternal species. The seeds of the amphidiploids have traces of silver-like colour that is characteristic for *N. multivalvis*.

F_1-hybrids *N. multivalvis* × *N. suaveolens* have very irregular meiosis. They have usually only univalents or one bivalent during the first meiotic metaphase. Occasionally two bivalents were also observed, but pollen mother cells with two bivalents rarely occurred.

The reduction division in the amphidiploids is quite regular. We observed very rarely one polyvalent (trivalent) and one or two univalents. Lagging chromosomes on the spindle rarely occurred. The plants formed about 88 per. cent viable pollen.

This high constancy can be explained by almost complete absence of bivalents in F_1-hybrids and polyvalents and univalents in the amphidiploids. The appearance usually of 40 bivalents in the first metaphase which originate from autosynthetic association between *multivalvis* homologous chromosomes as well as between *suaveolens* homologous chromosomes is an ultimate sequence from the absence of homology between *multivalvis* and *suaveolens* chromosomes (almost complete absence of allosyndesis).

It should be mentioned here that *N. multivalvis* is an American species while *N. suaveolens* is an Australian species; hence the amphidiploid form combines the characters of an American and an Australian species into a fertile plant.

An amphidiploid plant in crossing Australian and American species was produced by Goodspeed (1933). He produced it from *N. suaveolens* × *N. Bigelovii* hybrid and called it *Nicotiana disualovii* (see Elvers, 1934).

We are greatly indebted to Z. Vishemirskaya, M. Linden and V. Botcharova for help throughout this work.

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FIG. 2.—M—*Nicotiana multivalvis*; H—amphidiploid hybrid *N. multivalvis* × *N. suaveolens*; S—*N. suaveolens*.

OBSERVATIONS ON THE SWARMING HABITS AND LUNAR PERIODICITY OF *PLATYNEREIS* SP. FROM THE MADRAS HARBOUR.

By R. Gopala Aiyar, M.A., M.Sc.,
and
N. Kesava Panikkar, M.A., M.Sc.
(*From the University Zoological Laboratory, Madras.*)

Received April 20, 1937.

Introduction.

While collecting specimens from the Madras Harbour on a new moon night during March 1936, a number of worms were found swarming on the surface, causing brilliant phosphorescence in the water. Several specimens of this worm were collected with a tow-net, and later examined in the laboratory, which showed that they were heteronereid phases of *Platynereis* sp., a Polychæte belonging to the family Nereidæ. Since the phenomenon of swarming is of considerable biological interest, regular observations were subsequently made, and a periodicity in relation to lunar phases was discovered in the behaviour of these Polychætes. So far as the authors are aware, periodic swarming of Heteronereids has not hitherto been recorded from the Indian coasts, though in a Syllid, Gravely (1927) once observed swarming and phosphorescent display at Krusadi Islands. The following account of the Heteronereid stage and the lunar periodicity in the swarming habits of this species is submitted with the hope that this will lead to further observations on similar phenomena that may occur also in other localities on the Indian coasts.

Literature.

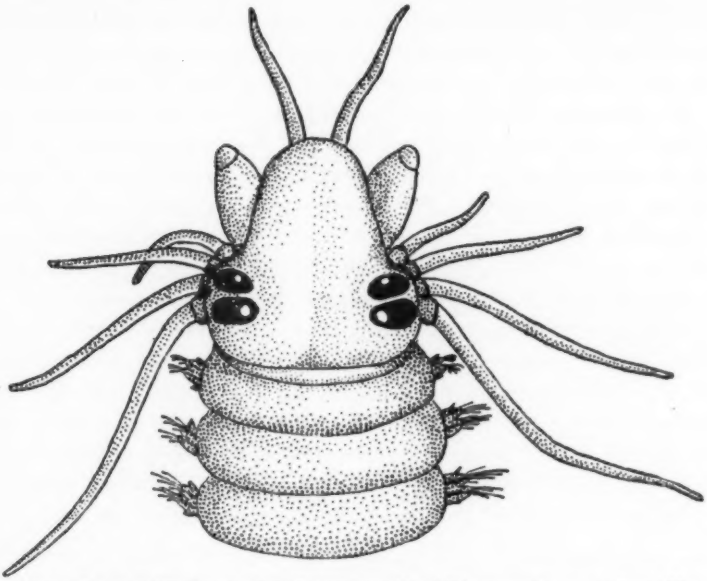
Swarming behaviour of Polychætes has received the attention of several investigators in Europe and America ; many Nereids and Eunicids are known to swim at the water surface in large numbers, when they are sexually mature and are ready to discharge their reproductive elements. Each worm is believed to take part in swarming only once, as in most cases it dies after the extrusion of the ova or spermatozoa as the case may be. Most of the recorded instances of swarming take place during the night ; but instances of swarming during daytime are not unknown (Verrill, 1873 ; Herpin, 1928). The swarming may be annual, occurring only once in the year, and that for a

very restricted period, or it may occur several times during the year, between definite intervals. Thus Mayer (1908) found that the Atlantic Palolo (*Eunice fucata*) swarms only once in the year, the annual breeding season being only one to six days' duration, occurring in the third quarter of the moon between June and July. Izuka (1903), Friedlander (1898) and Horst (1905) have described a somewhat similar behaviour in three other species, *Ceratocephale osawai* (Japanese Palolo), *Eunice viridis* (Pacific Palolo) and *Lysidice oele* (Wawo of Malay Archipelago) respectively. While in the Eunicids the reproductive period is more or less annual, in many of the Nereids, the reproductive season extends for a considerable part of the year, during which successive swarms take place after regular intervals. Hempelmann (1911) found the Heteronereids of *Platynereis dumerilii* swarming at Naples from the month of October to May, during the first and third quarter of the moon. At Woods Hole, both *Nereis limbata* (Lillie and Just, 1913) and *Platynereis megalops* (Just, 1914) are known to swarm regularly, the first especially for a fairly long period. In Japan, another Nereid, *Nereis japonica* swarms in large numbers during December (Izuka, 1906). Numerous other accounts of Nereids are given by Gravier (1901, 1923), Fage (1923, 1926), Fage and Legendre (1923), Rene Herpin (1923, 1928, 1929), Horst (1911), Ranzi (1931) and Gallien (1936) ; of Eunicids by Woodworth (1907), Whitmee (1875) and Treadwell (1909) ; and of Syllids by Fraser (1915), Galloway and Welch (1911), Herpin (1924) and Potts (1913). Amongst these, the only account of swarming of Nereids from the Indo-Malayan coasts is that of Horst (1911) on *Platynereis polyscalma* (*vide* Fauvel, 1932 for synonymy of the species). Fauvel (1932) in his report of the Annelida Polychæta of the Indian Museum, mentions that several epitokous individuals of *Platynereis polyscalma* have been collected from the water surface at Nankuari Harbour, and this is a sure indication that swarming of this species takes place in this locality.

Description of Heteronereids.

General.—The worms are reddish-brown when alive and are usually 3 to 5 cm. long. Most of the specimens collected were transparent since the body cavity was empty, the germ cells having been shed. The body of the worms consists of an anterior unspecialized part and a posterior and longer epitokous region with highly flattened parapodia bearing large oar-shaped setæ.

Head.—The head is prominent and the prostomium is obtusely rounded. There are two pairs of eyes ; they are considerably enlarged and are provided with lenses. The two pairs are situated quite close, but they do not touch. All the eyes are alike in structure, but the posterior pair is slightly the larger.



TEXT-FIG. 1.—The head of the heteronereid of *Platynereis* sp. × about 20.

The eyes do not extend to the ventral surface and are not seen in a ventral view of the worm. In this respect, these specimens differ from *Platynereis polyscalma* and certain other species. The palps are present, but are not quite so prominent as in most heteronereids. The basal joints are swollen and are much longer than the terminal ones. All the four tentacular cirri are present in some individuals, but as they break away easily, most of the specimens examined do not have the full quota of cirri. The longest of them normally reaches the base of the fifth parapodium. Annulations are not visible on the tentacular cirri. The first or the uppermost pair is the smallest and reaches to the base of the second parapodium.

The number of segments observed in six typical specimens is given in the following table:—

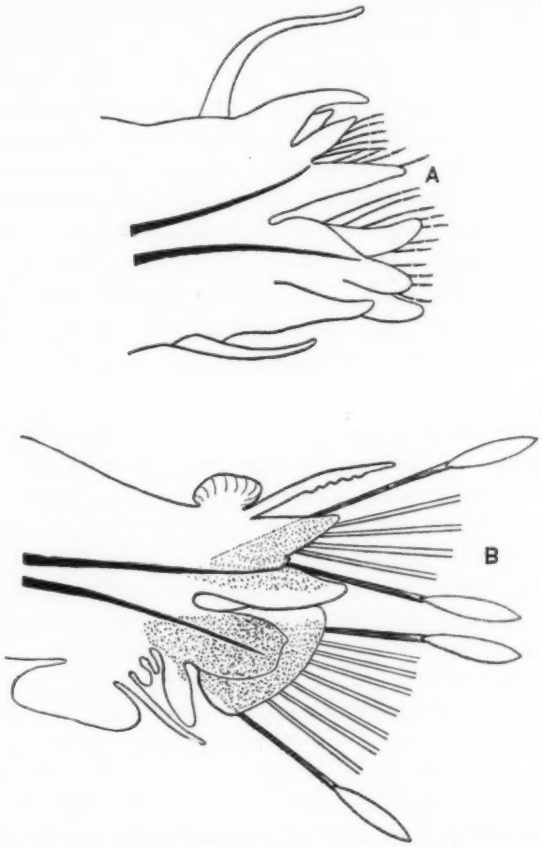
Number of worm	Total number of segments	Anterior atokous part	Posterior epitokous part
1	56	25	31
2	60	20	40
3	67	22	45
4	71	21	50
5	85	23	62
6	132	22	110

The Atokous Region.—The anterior atokous region usually extends to 20th–25th segment. As mentioned previously, the parapodia are unspecialized here, and are insignificant as compared with those that follow in the epitokous part. The structure of the tenth parapodium which may be taken as more or less typical for this region, is as follows. The dorsal cirrus is long and exceeds the length of the dorsal and ventral lobes of the parapodium. It is slender, arched and not crenulated. The dorsal lobe bears three ligules and a single aciculum with 8–10 homogomph setæ. The terminal portions of the blades are minutely serrated. Of the three ligules, the middle one is the smallest. The corresponding three ligules of the ventral lobe of the parapodium form a cluster with their tips standing widely apart. The dorsal part of the ventral setigerous bundle consists entirely of homogomph setæ with their blades stouter than the corresponding ones of the dorsal lobe; and the edges of the blades are coarsely serrated. The setæ are fewer in number in the ventral fascicle of the lobe, being about 7–8, and they consist entirely of heterogomphs, with shorter and coarsely serrated blades. The ventral cirrus stands apart from the rest of the parapodium.

The Epitokous Region.—There is considerable difference in the structure of the parapodia of the different portions of the epitokous region. The parapodia from the 2nd, 80th and 110th segments after the commencement of the epitokous transformation are described below from a complete worm.

In the second segment, the dorsal ligule is broadly lanceolate. Into the base of the dorsal lobe the aciculum projects, and the lobe is longer than the dorsal ligule. Lamellæ are not found at the base of the ligule. The dorsal cirrus of this segment and those of the few nearby are not crenulated. The lower half of the parapodium consists of two parts: a well-developed ventral lobe, and extending beyond it, a heart-shaped lamella. The ventral ligule is quite distinct and lanceolate in shape. No appendage is seen at the dorsal side of its base. At the base of the ventral cirrus and especially on its dorsal aspect, there are two leaf-like processes. Ventrally, there is a lobed cordate structure, the bifurcated lamella of some authors. So far as the setæ are concerned, there is not much difference between those occurring in the upper and lower bundles. The blades start almost from the bases of the lobes, differing from what Gravier (1923) and Horst (1911) noted for other Heteronereids.

In the parapodium from the 80th segment, which is about the middle of the epitokous region, the dorsal cirrus is distinctly crenulate, with five papillæ on the inferior border. The cirrus projects beyond the notopodium



TEXT-FIG. 2.—Drawings of parapodia of the heteronereid of *Platynereis* sp.
A.—Parapodium of the 10th segment from the atokous region. × 50.
B.—Parapodium from the 80th segment, from about the middle of the epitokous region. × 40.

for about a third of its length. Dorsal to its base is seen a rounded lamella. The ventral cirrus has a bifurcated process on the upper side, above which the ventral ligule projects freely. There is a large ear-shaped lamella below the ventral cirrus. This is much bigger than the corresponding one described for the 2nd segment of the epitokous region.

The parapodium of the 110th segment is very similar to that of the 80th. Only four papillæ are noticed on the inferior border of the dorsal cirrus. The ventral lobe is smaller, and the ligule found here is almost as large as the one on the dorsal. Ventral cirrus carries a bifurcated process at the

upper part of its base and smaller leaf-like structures are present on the lower side of the base. The setæ are less numerous than in the 80th segment, but the structure of the blades does not present any notable difference.

The oar-shaped setæ of the epitokous region have the usual complex structure which has been described by many authors. The terminal blade has got minute serrations on its inferior border; but the border is not at all conspicuously pectinate unlike what occurs in *Platynereis polyscalma* (Fauvel, 1932; Horst, 1911).

The pygidium is not seen in most of the examples as the worms are mostly incomplete, the tails having broken away. In one specimen, two anal cirri were observed. Near the tail region, the natatory setæ decrease very much; but the ventral and dorsal cirri of the parapodia are quite prominent.

Armature of the Pharynx and the Systematic Position of the Species.—The pharyngeal armature consists of separate horny paragnaths, of which all the groups are not represented. Those present are arranged in pectinate rows of minute denticles. All the dorsal groups of the maxillary ring are missing, as also some of the dorsal groups belonging to the oral ring. These features are in agreement with Fauvel's description of the genus *Platynereis* (Fauvel, 1932); but the present form cannot be definitely assigned to any of the species of the genus known from the Indian coasts. The species recorded by Fauvel from India are (1) *P. dumerilii*, (2) *P. abnormis* and (3) *P. polyscalma*. In *P. abnormis*, there is a very long dorsal cirrus on the 7th setigerous segment, while in the two other species, the 7th dorsal cirrus is normal. In the absence of the long dorsal cirrus, the species is in agreement with *P. dumerilii* and *P. polyscalma*. In the latter, the oar-shaped setæ are boldly pectinate, while it is not so in our specimens. Thus, of these three species, the one agreeing closely with the present form is *P. dumerilii*; but there is a notable difference in that the tentacular cirri extend only up to the 5th segment in the present species, while in *P. dumerilii* they are much longer, extending to segments 10 to 15.

Notes on Internal Organization.—Three of the specimens collected were sectioned after fixation in Bouin's fluid. In all the examples, the alimentary canal was much degenerate and without any food contents. The body-cavity in all of them was empty as the germ cells had been shed more or less completely. However, traces of spermatozoa were found in the body-cavity of all the specimens sectioned. Further, a few live specimens teased and examined under the microscope revealed the presence of spermatozoa. This confirms that the specimens collected are mostly males, a fact also suggested

by the crenulated nature of the dorsal cirri of the epitokous region. With the probable exception of two individuals, all the specimens collected by us on all the occasions when the swarming took place are males.

The body-wall has essentially the same structure as in other Polychætes, but its thickness is considerably reduced here. The longitudinal muscle bands that are so prominent and characteristic of atokous Nereids are rather insignificant here as they are reduced to thin bands. The alimentary canal occupies only a very small part of the body-cavity of the worm. Sections clearly show that the walls of the alimentary tract have begun to undergo degeneration ; and a number of loose cells, which originally belonged to the gut-wall, are found in the lumen of the intestine.

Swarming.

The Environment.—The swarming of Heteronereis reported in this paper is based upon observations made inside the Madras Harbour for a period of one year from March 1936. The harbour is an artificial enclosure of about a quarter of a square mile in area. As compared to the outside sea, the water is always calm inside the harbour, the action of the waves and currents being brought to a minimum by the embankments that run on all sides excepting for a narrow entrance. The salinity records for the Bay of Bengal* near Madras range from 30·00 to 34·50 per mille within the course of the year ; inside the harbour, there is reason to believe that the salinity of the water is slightly less than the above figures, especially during the rainy weather. The temperature of the sea water normally varies from 25 to 30° C. The usual tidal range is about 2 to 3½ feet. The embankments, the boulders of the wharfs, and the buoys scattered about here and there, support a rich littoral fauna consisting of numerous sponges, Cœlenterates, Polychætes, Molluscs, Crustaceans, Echinoderms and Ascidians. Nereids in their atokous phases are often observed amongst the scrapings from the boulders.

Swarming Periods.—All instances of swarming observed by us occurred between 7 and 10 p.m. on the new moon days and a day or two prior to or after. The time corresponds to the period of maximum high tide for the particular day (compare Table I). On every occasion, the sea was calm and external factors like powerful wind, or rain were not in operation. There was intense phosphorescence in the water caused by the Dinoflagellate *Noctiluca miliaris* occurring in large numbers on the water surface, and emitting splashes of phosphorescence at every disturbance in the water. The

*Sewell, R. B. S., *Mem. Asiat. Soc. Bengal*, 9.

luminescence observed during swarming of these Nereids is really caused by Noctilucæ, reacting to similar disturbances in the water, produced by the swimming Polychætes. Unlike the Syllids, the worms themselves do not emit light.

Behaviour of Worms.—The behaviour of swarming individuals is as follows. The onset of the phenomenon is evidenced by an occasional worm or two darting through the water surface here and there by about 7 P.M. Other individuals follow, and in about half an hour's time, numerous worms come up to the surface and swim about, leaving several trails of phosphorescence in the water. Each worm swims rapidly in a spiral manner. When the swarming is at its height, numerous trails of phosphorescence are found, indicating intense activity on the part of the worms. Their movement through the water is so quick that it is often difficult to catch them in the nets. The number of individuals taking part in swarming in the Madras Harbour is by no means high, when we consider the records for *Nereis japonica* (Izuka, 1908), *Neries limbata* (Lillie and Just, 1913), etc. In these cases, the swarming is so intense that thousands of worms come up to the surface, and several worms are obtained in any scoop of water taken from the surface. On the other hand, we could collect worms only after repeated attempts with the tow-net.

Most of the swarming individuals are males and females are rather rare among the specimens collected by us. The large excess in number of males over females has been mentioned by most authors. Lillie and Just (1913) record that the females come only some time after the males have come to the surface. From a series of experiments these authors conclude that a chemical stimulus from swarming males is necessary for the females to spawn. We have not so far observed actual sexual congress between the swarming males and females, which Just (1914) has described as occurring in *Platynereis megalops*.

Dead worms with body-cavities empty have been obtained the morning after the night of swarming.

Records of Observations.—The swarming was first noticed on the 22nd of March 1936, which was a new moon day. The display commenced at about 7-30 P.M. and lasted till 9-00 P.M., and a large number of worms took part. A few individuals were observed on the next day at about the same hour as on the previous day. No display occurred on the next day and from now onwards, the phenomenon had completely ceased to take place. Observations were made on every day till the next full moon ; and there was no recurrence of swarming either during the full moon or the succeeding

new moon. Thereafter, regular observations were continued on all new moon and full moon days as well as on the nights before and after every full and new moon.

The phenomenon did not take place during the three full moons and two new moons after the first swarming. The next occasion on which swarming was noticed was the third new moon after the one in March. On June 18th (new moon day) a weak swarming was noticed; but on the next day there was intense swarming between 8-00 and 9-45 P.M. On the next day also a few worms were observed but their number was considerably smaller than that on the 18th. The succeeding days as well as the lunar phases during July and August passed off without any display.

For a third time, the phenomenon was repeated on September 15th, the day before the new moon. A large number of worms swarmed on this day between 8-00 to 9-30 P.M. Swarming was fairly intense on the next day as well, but commenced only by about 8-15 P.M. and lasted till 10-00 P.M. On the 17th of September, there was a weak display.

Swarming was not observed on any other occasion than those given above, though repeated observations were made from March to December 1936. The intervals between the dates on which the display occurred would suggest the probability of the phenomenon occurring during the new moon in December. Though we hoped to find the Polychætes swarming, none were noticed on this occasion. Careful observations were made on the new moon day (December 13th) as well as the days prior and after; on none of these days was there any trace of display. There was no phosphorescence owing to the almost complete absence of Noctilucæ during this season. The sea was very rough and even inside the harbour, the water surface was very agitated. Several collections were made with the tow-net and not a single Polychæte could be obtained. At present we are not in a position to say whether the absence of swarming on this occasion was due to the roughness of the sea or to the fact that the annual swarming had stopped with the September display. The fact that there is a definite period of swarming in all known instances among Polychætes, would point to the latter explanation being the more probable. If so the Madras species of *Platynereis* swarms only from March to September. In the year, 1937, swarming again occurred during the new moon in the month of March.

The records of swarming along with the condition of the tide and the duration of the display are given in the accompanying table. The periods of swarming and the dates would clearly indicate the following facts:—

1. Swarming takes place only during new moon, the preceding and the succeeding one or two nights in every case, *i.e.*, during three days on every occasion; the time of swarming corresponds to the time of maximum high tide during the particular night.
2. The phenomenon does not take place on all new moons; but a regular periodicity is seen in that three lunar months elapse between any two successive swarms.
3. Slight variations may occur in that the highest intensity of the display may be reached on the new moon day, the day previous or the day after.

Swarming of Platynereis *in the Madras Harbour.*
Statement of Records for the Year 1936.

Date 1936	Phase of Moon	Time of maximum high tide* hr. min.	Tidal range* ft.	Hour and duration of swarms	Intensity of swarms
March 22	New Moon Day	20-03	3·4	19-20—21-00	Intense
,, 23		20-43	3·5	,, ,,	Sparse
June 18	New Moon Day	19-45	2·9	20-00—21-45	Sparse
,, 19		20-34	2.9	,, ,,	Intense
,, 20		21-18	2·9	,, ,,	Very sparse
Sept. 15		20-26	3·3	20-00—21-30	Intense
,, 16	New Moon Day	20-50	3·4	20-15—22-00	Fairly intense
,, 17		21-17	3·5	,, ,,	Sparse

* " Tide Tables of the Indian Ocean for the year 1936," Survey of India Publication.

Discussion.

The belief in the influence of the moon over reproductive rhythms in organisms is several centuries old and is current to this day in different parts of the world. Whatever the rôle of the moon in causing these rhythms, the occurrence of sexual periodicities in relation to definite lunar cycles has been established beyond doubt in a number of organisms as a result of many investigations by recent workers. The list given by Amirthalingam (1928) includes a large number of species, both plant and animal, exhibiting lunar periodicities in reproduction. The following tabulated statement gives the details of lunar periodicities among the known instances of Polychætes, which include species that swarm as well as those that do not. It will be

Lunar Periodicity in Polychætes.

Species	Author and Date	Place	Time	Remarks
Eunice viridis .. .. (Pacific Palolo)	Whitmee 1875 Freidlander 1898	Samoa	October to November	Third quarter of moon
Eunice fucata .. .. (Atlantic Palolo)	Mayer 1908	Tortugas, Florida	June to July	Third quarter of moon
Lysidice œle .. .. (Wawo)	Horst 1905	Malay Archipelago	March to April	Second and third nights after full moon
Ceratocephale osawai .. (Japanese Palolo)	Izuka 1903	Japan	October to November	Swarming at both full and new moon
Nereis limbata	Lillie and Just 1913	Woods Hole	June to September	Full moon and third quarter
Neresis japonica.. ..	Izuka 1908	Japan	December	New or full moon in December
Platynereis dumerilii ..	Hempelmann 1911	Naples	October to May	First and third quarter. None at full and new moon
Platynereis megalops ..	Just 1914	Woods Hole	July to August	Between full and new moon and none between new and full moon
Eulalia punctifera ..	Fage and Legendre 1926	Concarneau		Third quarter of moon
Odontosyllis enopla ..	Galloway and Welch 1911	Flatts Isles		Third quarter of moon
Amphitrite ornata ..	Scott, J. W. 1904	Woods Hole	July to August	Within two days of full and new moon. Probably associated with tides

* Isolated instances of swarming recorded by certain authors are not included in this list.

seen from the list that amongst the recorded instances, there is no Polychæte that swarms exclusively during the new moon; and the behaviour of *Platynereis* sp. described in this paper is in this respect exceptional. *Ceratocephale osawai* and *Amphitrite ornata* are the two species that spawn during the new moon; but both these species are bilunar, and reproductive activity occurs during full moon (or within two days) as well as the new moon. The Palolo may be left out of consideration from the following account of comparison as swarming among them is not a periodical phenomenon as in most Nereids, though the annual swarming by itself is in relation with

some particular lunar phase. Amongst the Nereids, *Nereis japonica* swarms either during new or full moon ; the swarming period is almost as short as in the Palolos, there being at most only two " runs ". The reproductive period of *Platynereis megalops* extends from July to August, the swarming of Heteronereids taking place during the dark phases of the moon. Only in *Nersis limbata* and *Platynereis dumerilii* do the swarming periods extend for a long time of the year ; and the Madras species is comparable to them in that the reproductive season extends from March to September. *Nereis limbata*, however, shows a marked intensity towards the full moon, while swarming during full and new moons does not take in *Platynereis dumerilii*. Further, an important point to be emphasized here is the remarkably long intervals between the swarms observed in the present form. In no Nereid, or Palolo for that matter, do we find more than one lunar month elapsing between one swarm and the next one in the same series, while in this instance, an appreciably long interval of three lunar months is observed between the successive swarms.

The fact that only mature individuals swarm, and that they die after the extrusion of the sexual products prove beyond doubt that the dehiscence of sexual products is the object of this peculiar behaviour among the Polychætes. Other factors like food, etc., noted for some Crustaceans (*cf.* Wheeler and Brown, 1936) are not relevant to the swarming of Nereids and Eunicids. But how may the relation of the reproductive rhythms to the lunar phases be explained ? The conditions necessary if swarming should take place as judged by the records of our observations appear to be (1) high tide, (2) absence of light, (3) relatively calm weather, and (4) coincidence of these factors with the particular lunar phase. Temperature, currents and salinity do not seem to have any influence on the swarming as the variations are slight and do not have any correlation with the lunar cycles. We have pointed out that swarming of the Madras species does not take place always, even if all the above conditions prevail. The same environmental conditions are practically repeated during every new moon, but swarming occurs only on some selected occasions. This strongly suggests that what determines swarming is not primarily the stimulus of the environment, nor the direct influence of the moon, but is a factor entirely depending upon the swarming individuals. The development and dehiscence of sexual products is in most organisms a periodic phenomenon ; in the Polychætes that swarm, each individual assumes the epitokous stage only during the last phase of its life ; when the sexual products are fully mature, the worm swarms, evacuates the genital contents and finally dies. If swarming should occur between regular intervals, successive batches of worms should be involved ;

and the interval of three lunar months observed after one swarming till the next one, is the time taken by the next batch of worms to attain that right stage of sexual maturity prior to swarming. The absence of display during the intervening optimum lunar periods may be explained as being due to the paucity of sexually mature individuals at this time.

As in other instances of lunar periodicity, the definite preference shown by the worms for spawning at a particular phase of the moon over others, cannot adequately be explained. Lillie and Just (1913) make the assumption that the maturity of the animals is dependent on some relation of the life-history to the phases of the moon involving, probably through lunar tidal variation, rhythmic alternation in the condition of nutrition. Fox (1923) has suggested that an external rhythmic change in the environment synchronous with lunar periods, might influence the developing germ cells, or might have a trigger-like action on the gonads already filled with genital products (*vide* Galtsoff, 1935). The duration of the daily period of illumination has also been suggested by Fox (1930) as a possible cause.* It cannot be doubted that lunar periodicity in oranisms is caused by different factors; and species exhibiting the same merits individual consideration. For some species, it may be purely tidal, as conclusively shown by Cark (1925) for the Atherine fish *Leuresthes tenuis*. The chief factor determining periodicity in any species is the life history of its gonads, but the environment may suitably aid in the final liberation of the genital contents. The high tide, the calmness of the sea, the absence of moonlight may all be contributory causes to which sexually mature worms may show some response; the cumulative effect of all these may be the stimulus that induces them to swarm and discharge their genital products.

One point which needs further elucidation in explaining the periodicity in the present form is the period of life of the worms. The conclusion arrived at before, that distinct batches of worms swarm periodically with an interval of three lunar months after every case, raises the question whether the worms taking part in any one swarm represent individuals developed from eggs fertilized during the previous swarm or those developing after the corresponding swarm in the previous years. If the first alternative is the correct one, it would mean that these Nereids do not live for more than three months, their larval, atokous and epitokous phases taken in all. This is hardly credible, though it may be mentioned that the rate of growth of animals of the Madras Harbour is rather high as found by Mr. M. D. Paul (unpublished

* For recent literature on lunar periodicity in reproduction, *vide* Marshall, S. M., and Stephenson, T. A., "The Breeding of Reef Animals, Pt. 1.—The Corals," *Sci. Rep., Great Barrier Reef Expedition*, 1928–29, 1933, 3, No. 8.

observations). The absence of swarming during December is also not easily explained if this view is accepted, except that the display ought to have taken place, but did not actually occur owing to unsuitable weather. The second view which implies that the worms live for at least one year appears to be the more acceptable. Only continued and critical observations carried on for several years can definitely settle this point. It may finally be said that of the many factors that have been considered as giving rise to swarming for reproductive purposes, no single factor or group of factors may exclusively be considered to produce swarming. While it is true that environmental conditions play a large part in these phenomena, the fact that the individual animal may have something in its organization which enables it to slightly or wholly modify its response to environmental influences is often lost sight of. Upon the proper understanding of this relationship depends the way of solving problems of animal behaviour.

Acknowledgment.

We wish to thank Mr. M. D. Paul for bringing to our notice the occurrence of swarming in the Madras Harbour, and also for making field observations on many occasions.

Summary.

Heteronereids of *Platynereis* sp. swarm in the Madras Harbour during the new moon in the months of March, June and September. An interval of three lunar months is observed between successive swarms. Swarming takes place in the night, between 7 and 10 P.M. corresponding to the period of maximum high tide ; it may occur on the new moon night as well as on one night previous or after. The Heteronereids are described ; they appear to be different from the species of *Platynereis* already known from India. The behaviour of the worms during swarming, swarming periods and the records of observations are given in detail. The periodicity observed in this species is compared with other known instances, and the question of lunar periodicity in reproduction is discussed.

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OBSERVATIONS ON THE BREEDING HABITS AND DEVELOPMENT OF CERTAIN BRACKISH WATER FISHES OF ADYAR, MADRAS.

By S. Jones, B.A.

(From the University Zoological Research Laboratory, Madras.)

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(Communicated by Professor R. Gopala Aiyar, M.A., M.Sc.)

Introduction.

This work is part of a scheme proposed for the study of the brackish water fauna of the Adyar river. The Adyar backwater has an area of about 1 sq. mile lying about five miles to the south of Fort St. George. The water is shallow and practically stagnant during the greater part of the year except soon after the rains when connection is effected between the sea and the backwater, which are otherwise separated by a sand-bar. The temperature ranges from 25° C. to 33° C. and the salinity varies from 31 to 16 per mille.* This fall in salinity is really due to the influx of fresh water which the river brings after the bar is closed. The bottom is composed of loose mud with plenty of algal growth in the shallower regions. The only places where hard substrata are available for attachment of animals are near the Elphinstone Bridge and opposite the Theosophical Society Buildings where there is a stone embankment. The water here is rather deep and the place sufficiently protected for the young fishes to develop in fair safety. A comprehensive account of the Adyar backwater is given in a paper by Prof. R. Gopala Aiyar and Mr. N. Kesava Panikkar on the " Brackish Water Fauna of Madras " in course of preparation.

Very little attention has been paid so far to the study of the development of the brackish water fishes in India. The earlier works in this connection are those of Willey (1910 and 1911), Sundara Raj (1916), Bhattacharya (1916), Panikkar (1920) and Aiyar (1935) whose accounts are often incomplete.

Collections of eggs were made fairly regularly and observations were made on living material on the spot and after bringing them in clear water to the Laboratory. Care was taken to compare developmental stages obtained from the river and similar stages in the batches of eggs brought

* Subramaniam, M. K., *Jour. Roy. Micr. Soc.*, 1935, p. 13.

from there and allowed to develop in the Laboratory under suitable conditions. In the case of *Boleophthalmus boddærti* eggs were artificially fertilized in the Laboratory and development followed.

The breeding habits and development of *Acentrogobius viridipunctatus*, *Petroscirtes bhattacharyæ* and *Boleophthalmus boddærti* are made known for the first time though unfortunately that of the last mentioned one is still incomplete. The occurrence of external gills in *A. viridipunctatus* is very interesting and is made the subject of a separate paper. The cement organs of *Etroplus maculatus* have been studied in detail and are dealt with in another paper. In regard to *E. maculatus*, *E. suratensis*, *Aplocheilus melastigma* and *Panchax parvus* more complete developmental details are given.

Etroplus maculatus (Bloch).

The eggs of this form were first observed about the middle of August 1935 just after the first monsoon rains. The mother chooses the vertical edge of a stone or molluscan shell raised about a couple of inches above the bottom and lays a few eggs at a stretch allowing the male to fertilize them now and then. This is repeated, the male and female intermittently taking the responsibility of driving away other fishes which happen to come anywhere near. Panikkar (1920) has given a graphic account of the egg laying habit of *Etroplus suratensis* which tallies with my observations on *Etroplus maculatus*. Jerdon (1848)‡ and Sundara Raj (1916) have recorded their observations on the breeding habit of *E. maculatus* which were supplemented later by Panikkar.

No complete record exists about the development of *E. maculatus*. Sundara Raj (1916) and Panikkar (1920) have given rather an incomplete account of the larval development only. Same is the case with the account of *E. suratensis* by Panikkar who only gives a short life-history of this form.

The eggs of *E. maculatus* are peculiar in that the yolk is of a greyish brown colour, as also are the immature eggs in the ovary. This may help to serve as a sort of protective colouration as it harmonizes very much with that of the surroundings where they are generally deposited. They are oval in shape and measure 1·7 mm. in length and 1 mm. in breadth.

Development.— Fertilization seems to take place almost immediately after the eggs are laid and on examining a brood half an hour after oviposition most of them had reached the four-celled and eight-celled stages while

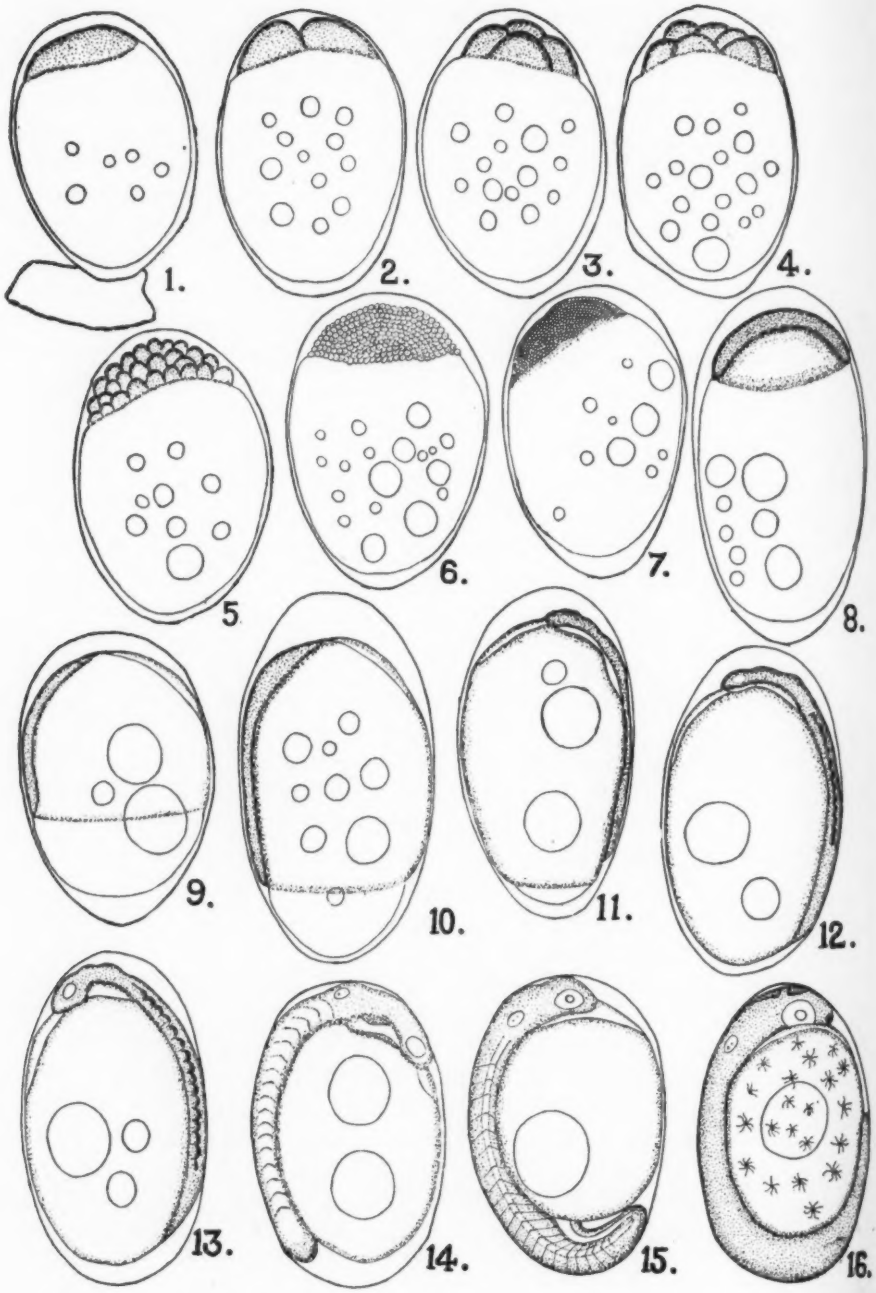
‡ It appears that the form *Etroplus coruchi*, the parental care of which Jerdon gives is really *E. maculatus*. Day (*Fauna of British India, Fishes*—II, p. 430) quotes Jerdon's observations with reference to *E. maculatus*.

some were in the two-celled stage and a few undivided. Evidently those that were undivided were the last to be laid or were the last to be fertilized. In these the protoplasm had collected together at the free end of the egg in the form of a blastodisc (Fig. 1) which is granular and devoid of any yolk. The undivided eggs within five minutes divided into two blastomers (Fig. 2) showing that the first division takes place about thirty-five minutes after fertilization. The yolk portion is full of small scattered oil globules which do not coalesce at this stage. (The large number of very small oil globules are not shown in the figures.)

Twenty minutes later, *i.e.*, 55 minutes after fertilization the two blastomeres divide to give rise to 4 (Fig. 3) and these, 35 minutes later, into 8 (Fig. 4). Further divisions were quite rapid and difficult to follow. Soon the whole embryonic area was converted into a cap-like cluster of small cells (Figs. 5 and 6). The blastoderm which remained like a cap at the top of the yolk gradually began to grow down towards one side (Fig. 7) and a thin layer of it began to spread over the surface of the yolk. The minute oil globules in the yolk coalesced and increased in size.

About 12 hours after oviposition the embryonic ridge is visible as a median thickening in the blastodermic area (Fig. 8). By this time the cells are greatly diminished in size. The thickened median ridge, which is the embryo proper, grows downwards along one side with the thin layer of blastoderm enveloping the yolk as a film (Fig. 9). As the embryo increases in length the head portion towards the top gets larger in size and the tail end grows downwards (Fig. 10). Twenty-eight hours after fertilization the contour of the dorsal surface of the embryo gets gradually distinct (Fig. 11). Two hours later the blastoderm completely envelopes the yolk mass which becomes internal (Fig. 12). Almost simultaneously with this the rudiments of the eyes make their appearance and the somites in the anterior portion of the trunk gradually become visible (Fig. 13).

The embryo rapidly grows in size. The eyes become larger. Four hours later, *i.e.*, about 38 hours after fertilization the auditory vesicles appear with two minute concretions and the tail end of the embryo grows broader. The first sign of movement sets in with faint and intermittent pulsation at the region of the heart (Fig. 14) when about 18 somites are formed. Forty-eight hours after fertilization the blood circulation is complete and the embryo wriggles inside the egg-membrane (Fig. 15). The tail portion of the embryo continues to grow round the yolk on which chromatophores appear (Fig. 16). The oil globules coalesce to form a large one. Eight hours later, *i.e.*, about 58 hours after fertilization, the tail of the animal almost completely encircles the yolk mass. On the morning of the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4th day when the embryo is 72 hours old hatching takes place. The egg-case breaks open above the dorsal side of the head and the tip of the tail is thrust out (Fig. 17). By vigorous movements of the tail the opening is widened with the result the tail projects out resembling the root of a germinating pea-seed and the larva moves about very actively at the bottom with the anterior portion of the body still within the egg capsule (Fig. 25, *Etroplus suratensis*).

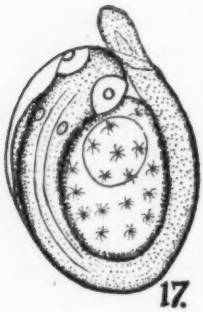
The larva comes suddenly out of the covering in the bent form. It remains quiescent for a couple of seconds as if taken aback by the abruptness of its exit; but loses no time to regain its activity and straightens up and moves with the head downwards at the bottom by the lashing movements of the tail.

Each larva measures 4 mm. in length. The head is attached to the yolk mass. The pigmentation of the eyes is so little that they are almost transparent. Mouth and anal openings are absent. Pectoral and pelvic fins are not developed. The median fins are all continuous and devoid of fin rays. The yolk has a single large oil globule and its surface is pigmented by closely arranged stellate chromatophores. The larva always rests at the bottom and never comes up to the surface.

A very interesting feature of this period is the presence of three pairs of cement glands on the head. Sundara Raj (1916) says that *E. maculatus* has two cement glands only and figures them as two star-shaped structures. Panikkar (1920) and Willey (1911) refer to their presence in the case of *E. suratensis*, but do not mention the number. Two pairs are dorsal, situated close together just above the eyes and the other pair is smaller and situated in front of the eyes just above the olfactory organs. Each gland is a small conical elevation with a concave depression at the top. The surface of the depression is lined with numerous mucous gland cells, the secretion from which fills the gland cavity and then oozes out continuously in the form of threads, which help to anchor the larvæ down to one place.

The larva assumes a slanting position with the head downwards and the ventral side up. It has a superficial resemblance to a tadpole. A large number of larvæ collect at one spot and the mucous threads help to hold them together (Fig. 20). When disturbed they detach themselves easily from the common mucous mass and move off; but gather in one place again.

On the second day (Fig. 21) pigmentation in the eye is greater though not complete. The rudiments of the pectoral fins appear. Sundara Raj



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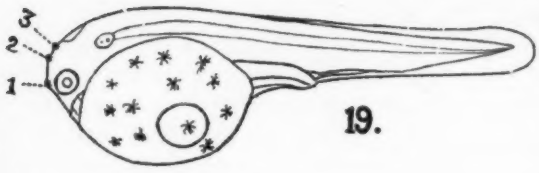

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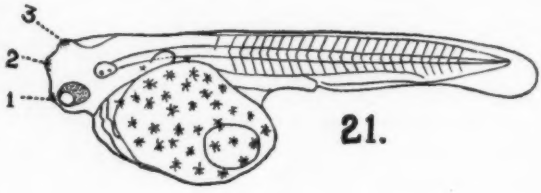

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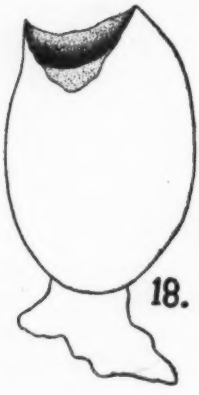

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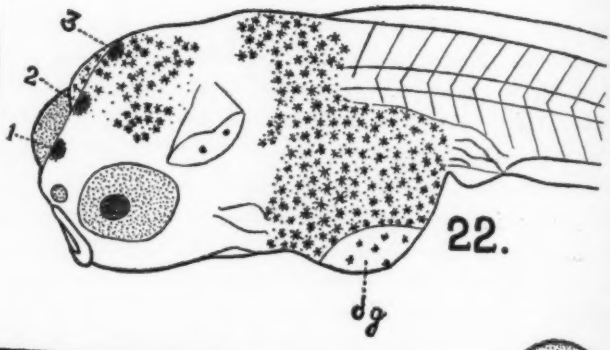

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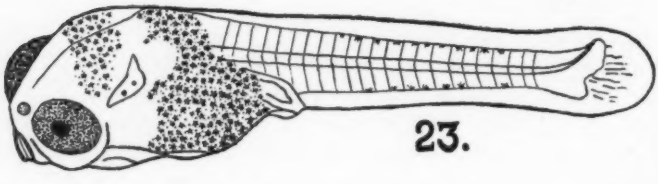

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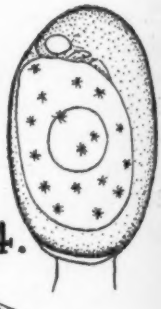

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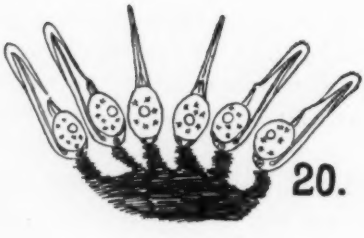

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(1916) has shown the heart dorsal to the yolk sac in the place of the pectoral fins. Chromatophores appear on the body above the yolk sac and on the dorsal side of the head.

On the third day the mouth and the gill slits are developed. The pectoral fins grow in size. Chromatophores increase on the dorsal side of the head and the nape. The yolk gets considerably reduced. The single oil globule persists.

On the fourth day (Fig. 22) the pectoral fins are capable of slow movement and along with this the cement glands become less active. Some of the larvæ lie on their sides and the rest with their dorsal side above. The greater part of the yolk is absorbed, the oil globule being the last to be consumed.

On the fifth day most of the larvæ come swimming up. The cement glands completely cease to function.

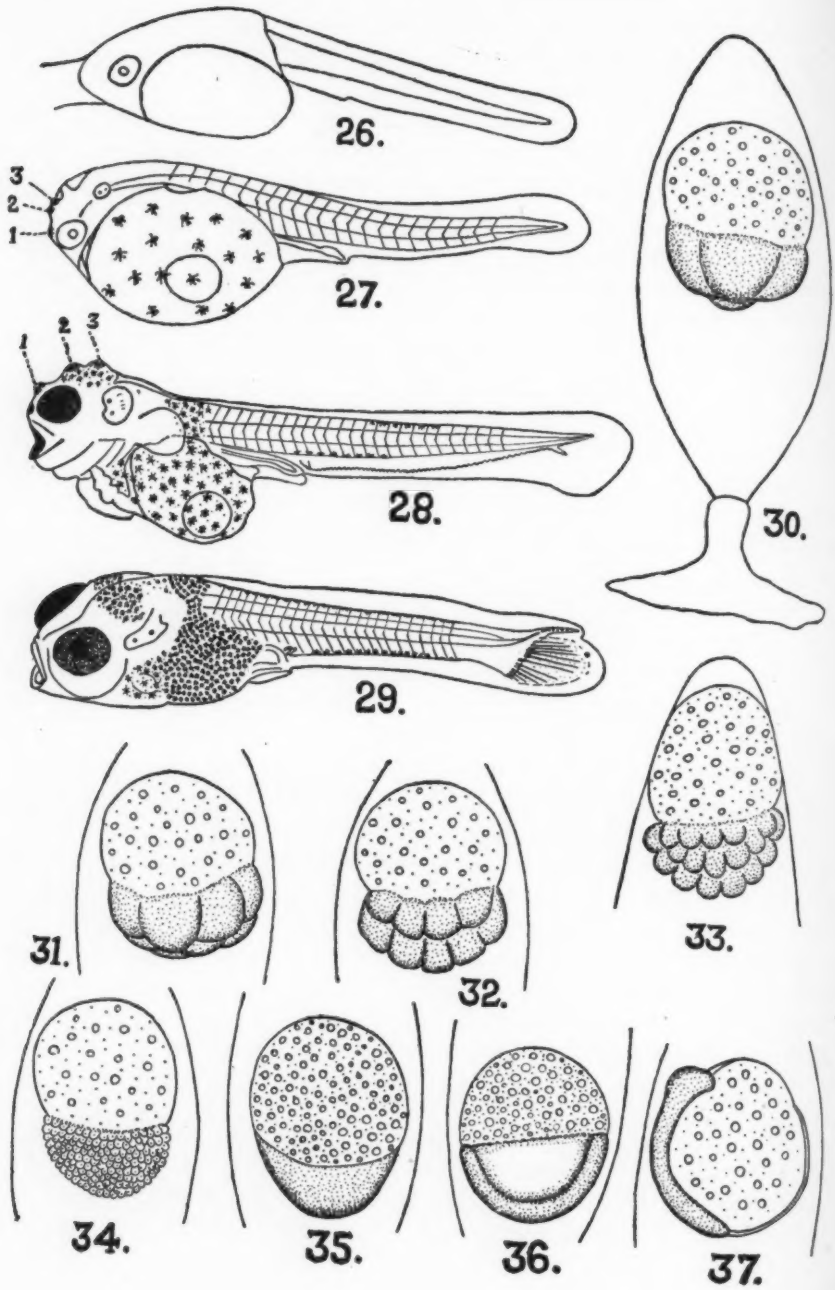
It has not been possible to see on what the young larva feeds. Fig. 23 shows a seven-day old larva. The caudal fin has become heterocercal and the anus is also formed. The caudal fin rays become visible on the tenth day.

In natural conditions, as soon as the time for hatching comes, the parents dig a small pit in the mud and transfer the eggs by their mouth into it. All the eggs are deposited in this manner and soon after hatching the larvæ are removed to fresh pits. This is continued till the larvæ are able to swim.

By the peculiar habits of the parents mentioned above these top heavy larvæ are virtually imprisoned in the pits. This helps to keep them together making the task of protection by the parents easy as otherwise their inability for independent movement will result in their destruction. Even after the larvæ are able to swim about independently and take food for themselves they are watched over by the parents for a considerably long time. During this period they do not come very near the surface. The slightest disturbance or apprehension of danger will send them shooting down to the bottom. The parents in their zeal to protect their brood will even snap at one's fingers as happened to the present writer many a time!

Etroplus suratensis (Bloch).

The breeding habits of *Etroplus suratensis* are similar to those of *E. maculatus*. I have seen the broods of these two forms fairly close together with continual war between their parents when one unconsciously trespasses the domain of the other. Willey (1911) was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the first to observe the nest and parental care of this form. Panikkar (1920) has given a detailed account of the breeding habits and development though he does not say anything relating to the early development within the egg-membrane. He does not figure any of the stages. The embryonic development of this form agrees so closely with that of *E. maculatus* in all details that it need not be described here.

Each egg is 2 mm. long (Fig. 24) and is similar to that of *E. maculatus* in shape and colour, though slightly larger in size. When the time for hatching comes, which is on the fourth day (*i.e.*, 72 hours after fertilization as in *E. maculatus*), the egg-case above the head of the embryo splits and the tip of the tail is thrust out (*E. maculatus*, Fig. 17). The opening is widened by the vigorous lashing movement of the tail till a good length of it is outside (Fig. 25). The larva moves about in this condition for some time till with a sudden effort the portion of the body inside the capsule turns through a semicircle, as a result of which the head comes at the base of the egg capsule (Fig. 26). Now the caudal portion of the larva is outside and it moves about just as the normal one with the capsule still around it, sometimes for quite a long time, till finally the opening gets sufficiently widened for the whole animal to come out.

The larva (Fig. 27) measures 4·3 mm. in length after hatching. The secretion of the cement glands is more profuse than in *E. maculatus*. Mouth, anus and gill slits are absent. The eyes are devoid of pigment.

The larvæ crowd together at the bottom with their ventral side up as in the case of *E. maculatus* (Fig. 20) continually vibrating their tails. When disturbed they move away along the bottom and crowd together again adopting a slanting position.

On the third day after hatching (Fig. 28) scattered chromatophores appear on the dorsal side of the head and nape. Mouth and gill slits are developed. The pigmentation of the eyes which began on the second day is completed by this time. The yolk persists with the oil globule in it. The pectoral fins are incapable of independent movement. The cement glands are still active.

On the fourth day the pectoral fins are capable of movement and simultaneously with this the cement glands become inactive. By the seventh day two concentrated patches of chromatophores are formed on the dorsal side of the head. Those on the body are more diffuse. The teeth are developed and a notch appears on the caudal fin. On the eighth day (Fig. 29) the anus is formed, the caudal fin shows fin rays and the animal begins to feed voraciously on algæ.

The larvæ of both *E. maculatus* and *E. suratensis* are very much alike except for the slightly larger size of the latter. The difference in the nature of the chromatophores is so little that it is very difficult to distinguish one from the other. The larvæ are watched over by the parents exactly as in *E. maculatus*.

When the bar opens and the sea water comes in, eggs are not laid. Developmental stages of the larvæ have not been met with when the bar was open. Very probably the parents go up the river into less saline conditions. This has been the case in the Cooum† also near the bar where an examination of catches of fishermen did not show the presence of any of these fishes when the bar was open.

Acentrogobius viridipunctatus (Day).

The eggs of this form could be obtained from Adyar throughout the year which shows that it is a perennial breeder. They are fairly common along the southern bank of Adyar (near the Theosophical Society Buildings) where there are plenty of stones. Eggs are generally found inside oyster shells and narrow crevices between stones. In all cases the male parent remains inside the shell or crevice as the case may be, aerating the eggs and guarding them against other egg-eating fishes. One male fish that was guarding the eggs was found with wounds probably inflicted by some other fish. The mother has never been found near the eggs except at the time of oviposition. Hence this is another instance of paternal parental care commonly seen among Gobies. This habit has been noted in the case of its congener, *Acentrogobius neilli* also, the development of which has been worked out in this Laboratory by Prof. Gopala Aiyar (1935). This form lays its eggs attached to thick clusters of algal filaments inside oyster shells, in the crevices between stones and on decaying wood. The eggs are smaller than those of *A. viridipunctatus* and there is some difference in the shape of the egg-case after fertilization. The early stages in the development of both the forms are very much alike.

For the purpose of laying eggs the mother usually chooses an oyster shell, the inner surface of which is quite clean. Newly submerged cement blocks, tiles and crevices of stones without any encrusting growth are also chosen for the purpose. Oviposition in all the cases I have come across, takes place at sunrise. At this time the urinogenital papilla of the female becomes long, stout and highly vascular forming a temporary ovipositor. A few eggs are laid at a time and the male which remains outside the shell

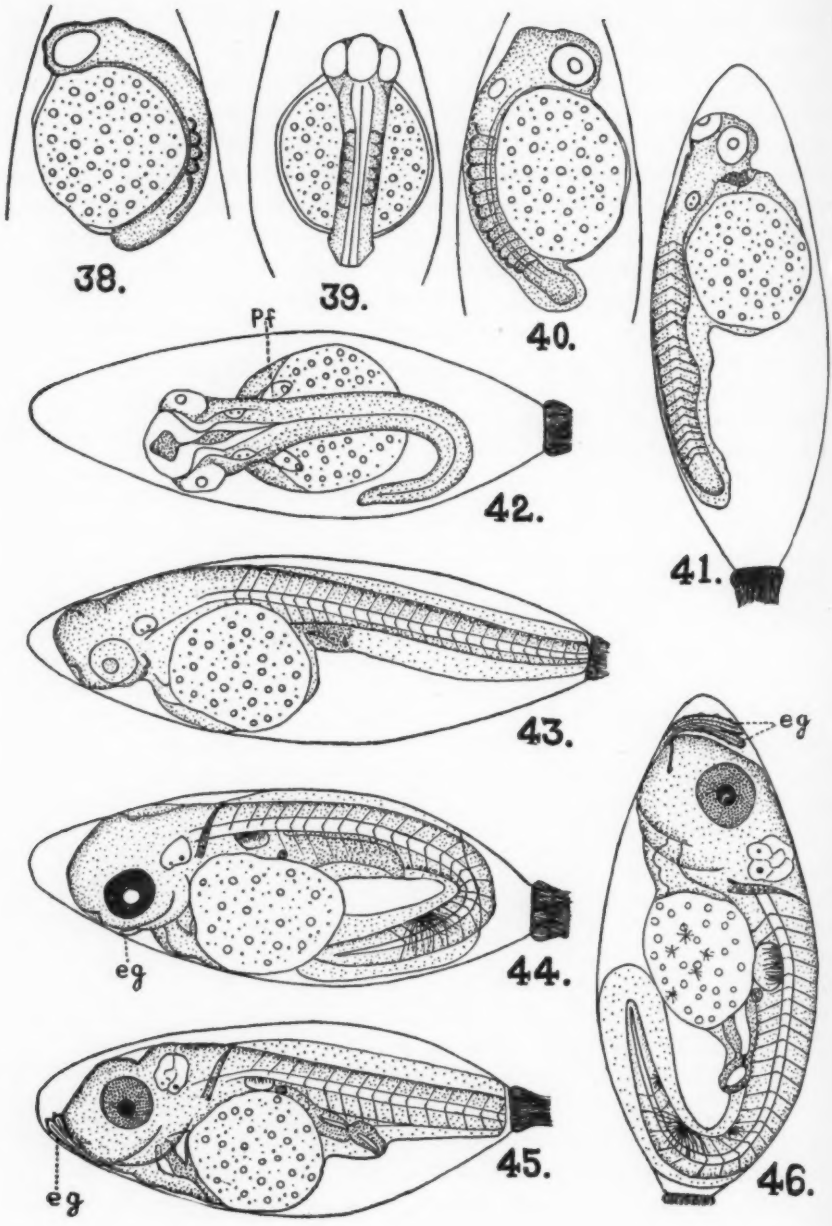
† A large drainage channel within the Madras City which is generally called the river Cooum.

till then goes inside and pours its milt over them and fertilizes them. These acts are repeated by the couple as a result of which immediately after oviposition one can see the eggs in varying degrees of development. After all the eggs are laid the female leaves the place and the male takes charge of them and remains inside the shell until the time of hatching. During short periods of captivity in the Laboratory aquarium the parents were seen to exhibit cannibalistic tendencies by devouring eggs and young larvæ.

An unfertilized egg is round and yellow in colour and measures 0·8 mm. with a ring of transparent threads that help to anchor it to the substratum as soon as it is laid. After fertilization the zona radiata swells up and it measures 2·4 mm. in length. The adhesive threads elongate giving rise to a hollow stalk. As many as three thousand eggs are laid at a time, though the number varies considerably. The eggs are arranged fairly close together without touching one another.

The earliest stage observed is an hour and a half after fertilization when four cells are formed (Fig. 30). A very large number of small oil globules are seen in the yolk which never coalesce to form a single one as in *A. neilli*. Twenty minutes later the next division into eight cells takes place (Fig. 31). Sixteen and thirty-two celled stages are reached two and two and a quarter of an hour respectively after fertilization (Figs. 32 and 33). Five hours after fertilization the blastomeres appear as a cluster of small cells (Fig. 34). The blastodisc is distinct when seven hours old (Fig. 35) and three hours later the embryonic ridge appears as shown in Fig. 36 as a medium thickening in the blastoderm, which spreads as a thin film round the yolk mass. When twelve hours old (Fig. 37) the head end becomes larger and the blastoderm completely envelopes the yolk mass. In the eighteen hours old embryo (Figs. 38 and 39) the neural groove is clear, the rudiments of the eyes are formed as thickenings of the ectoderm and somites appear at the posterior region. The optic and auditory vesicles are formed and the caudal end grows free, when the embryo is about twenty-four hours old (Fig. 40). In a thirty-six hours' old embryo (Fig. 41) the tail is free, the eyes are distinct and the heart can be seen as a tube between the head and the yolk mass. The heart begins to pulsate when about twenty-two somites are visible in the trunk. Meanwhile two concretions are formed in the auditory vesicles. When the embryo is about forty-eight hours old (Fig. 42) blood corpuscles are formed and shortly after, circulation begins. The animal now moves its tail gently inside the egg capsule.

When the embryo is about seventy-two hours old (Fig. 43) the important embryonic blood vessels are formed. The dorsal and anal fin folds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are formed. Pectoral fins can be seen as bud-like projections. In the eighty-four hours' old embryo the anterior end of the ventral aorta at the base of the hyoidean arch, where it divides into the two hyoidean arteries, pushes out the body surface to form the first common rudiment of the external gills.

Four Days' Old Embryo (Fig. 44).—The eyes turn dark and the pectoral fins become larger. The air bladder is formed. A large single chromatophore very characteristic of this form, appears ventrally at the middle of the tail portion. The external gill rudiment is longer with a notch at the tip.

Five Days' Old Embryo (Fig. 45).—The eyes begin to shine. The external gills reach as far as the mouth. The pectoral fins have become very large and are kept close to the body. The yolk gets considerably reduced. The notochord shows a tendency to bend upwards at the caudal end so as to give a heterocercal appearance. Peristaltic movement has begun in the intestine and the gall bladder can be seen as a pale yellowish green body.

Six Days' Old Embryo (Fig. 46).—The external gills grow round the snout and reach as far as the eyes and are now and then flicked by the animal. They are two, simple, non-pinnate structures partially united at the base. The very large hyoidean arteries constitute the vascular loops of the external gills. The gill slits are formed and the internal gill lamellæ have begun to develop as small buds. The pectoral fins are intermittently moved. The animal occasionally wriggles inside the zona radiata.

Hatching takes place when the embryo is five to seven days old, though six days is the usual time. If a larva does not come out after the seventh day it dies as is very commonly observed in the laboratory aquarium. External gills are poorly developed and are with defective blood supply in unhealthy embryos which seldom hatch out and survive. The egg-case breaks open at the distal end and the animal comes out head first. Simultaneously with the exit of the larva the external gills become non-functional and shrink up into small structures on the ventral side (Fig. 47). The larvæ can be extracted a few hours earlier without any apparent ill-effect. The force of a current of water pressed out of an ordinary pippette will bring out a fully developed larva.

The larva when it comes out is 3·3 mm. long and has a small quantity of unconsumed yolk as shown in Fig. 47, the surface of which is pigmented with stellate chromatophores. Mouth, anal opening and gill slits are present. The pectoral fins are devoid of fin rays. Between the yolk sac and the anus is a small pre-anal fin. The notochord is distinctly turned

dorsalwards (heterocercal) at the caudal end and the caudal fin is usually provided with about 10 fin rays. The auditory organ (Fig. 49) differs very much from that of *A. neilli* (Fig. 50). Between the tail end and the anus is a large conspicuous stellate chromatophore with a beautiful reddish brown colour. This chromatophore is very characteristic in this form, and the others in front and behind are much smaller and inconspicuous. The larva usually moves about at the bottom and rarely comes up to the surface.

On the second day all the yolk is consumed and the liver becomes visible. The shrunken external gills either disappear or remain as minute rudiments. By the third day all traces of the external gills are lost. The notochord near the anterior end gradually gets curved. Within a week in the Laboratory aquarium the larva develops thirteen fin rays in the caudal fin (Fig. 48). The rudiments of the dorsal and anal fin rays are also formed. No other appreciable change takes place other than an increase in size. It has not been possible to observe on what the animal feeds at this stage.

The young larva or the well-developed embryo of this form can be distinguished from that of *A. neilli* on account of the following characters :— The larger size, the difference in the structure of the auditory organs, the presence of external gills, the large conspicuous chromatophore on the ventral side of the trunk, the absence of any large oil globule in the yolk, the heterocercal fin and the early development of the caudal fin rays.

Before the opening of the bar at Adyar but some time after the rains start the eggs of this form could be obtained in fairly large numbers ; but afterwards only very seldom, though the adult specimens were found in abundance.

Boleophthalmus boddærti (Pallas).

This interesting mud-skipper is fairly common in all cool and miry situations east of the Elphinstone Bridge, Adyar. About the breeding habits of *Boleophthalmus boddærti* I could find no record of any definite observations except those by Harms.[1] According to him both *Periophthalmus* and *Boleophthalmus* are viviparous. Well-developed embryos, he says, were found within the ovary of the former which came out at the slightest pressure. He is not certain at what stage the young ones come out. A large number of females of *B. boddærti* from Adyar was examined throughout the year, but none of them showed any developing embryos in the ovary.

A thorough search was made for their eggs which having ended in failure, an effort was made to artificially fertilize the eggs, obtained by

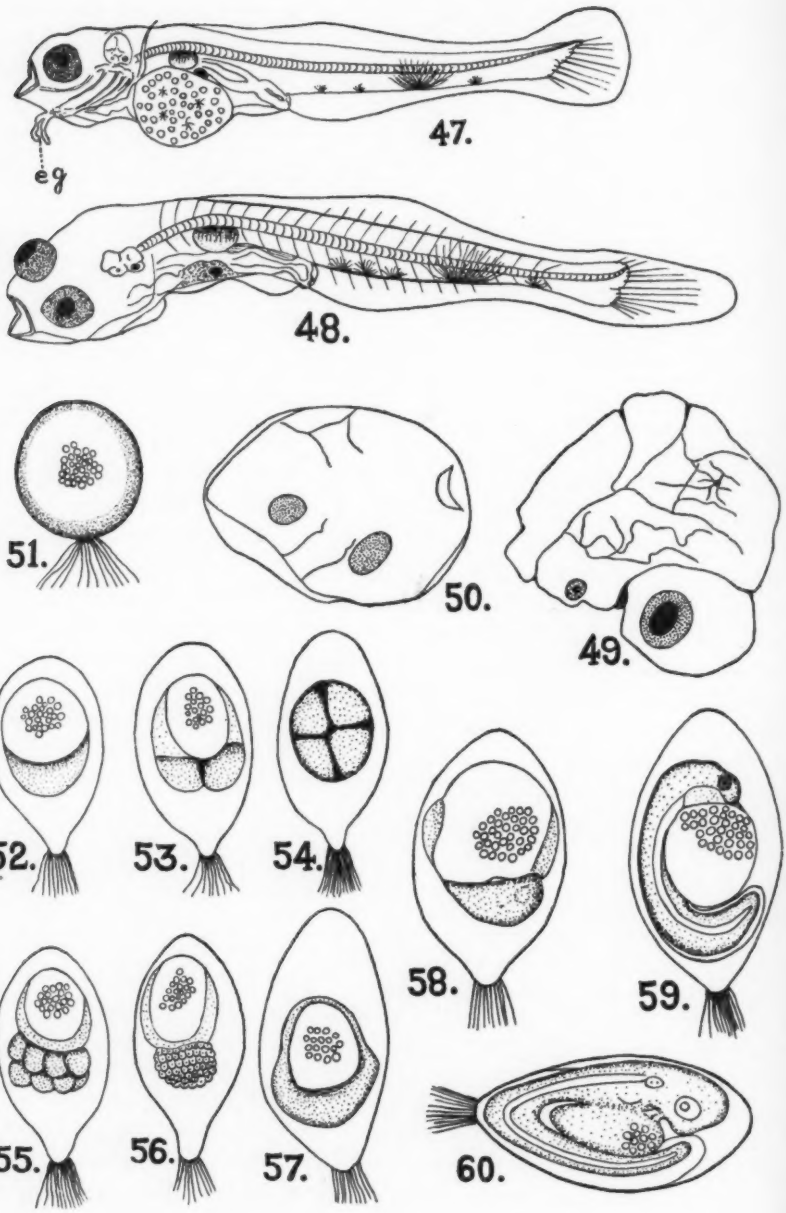
[1] Harms, J. W., *Zeit. f. Wiss. Zool. Bd.*, 1935, 146.

stripping the mature specimens. Except in a single instance the ovary was found to contain unripe ova. In the single specimen where ripe ova were present artificial fertilization was successful. Unfortunately as development proceeded the embryos began to die and though in many cases they were almost fully formed none of them hatched out. Apart from this, during the course of development many abnormalities were noted in a good number of embryos probably due to Laboratory conditions.

The ripe ovum before fertilization is 0·8 mm. in diameter. It is a round semi-transparent body with a cluster of adhesive threads sprouting from one side. The presence of anchoring threads show that as in *Acentrogobius* they should get attached to some surface as soon as they are extruded. It is very likely that the fertilization of the egg takes place externally. In the centre is a small group of oil globules. Soon after fertilization the zona radiata gradually swells out into an ovoid capsule and the protoplasm streams to the attached lower side of the egg to form a granular cap (Fig. 52). The attached side of the egg membrane from which the threads sprout, does not swell up in uniformity with the remaining portion with the result that the egg-case becomes almost club-shaped. The zona radiata attains a maximum length of 1.6 mm. Half an hour after fertilization the protoplasm divides into two to give rise to the two primary blastomeres (Fig. 53). This again divides into four (Fig. 54), eight (Fig. 55) and so on till three hours after fertilization they form a cap of closely packed cells (Fig. 56). Seven hours after fertilization a thin layer of blastoderm completely encircles the yolk and the embryo proper is formed in it as a median thickening (Fig. 57). When the embryo is seventeen hours old the head and the tail portions are distinct, though still attached to the yolk (Fig. 58). In a twenty-four hours' old embryo the caudal portion is free and the head comes to lie at the free end of the egg capsule (Fig. 59). Somites are formed in the trunk portion and a space is formed between the yolk and the head which locates the position of the heart. The optic vesicles are developed. The animal is incapable of any movement inside the zona radiata.

Forty-eight hours after fertilization (Fig. 60) the embryo is well developed with a considerably long tail which comes round the much diminished yolk and almost touches the head. The eyes are devoid of any pigment. The auditory vesicles have two minute concretions. The oil globules are much reduced in number. The embryonic circulation is well advanced and the animal wriggles actively inside the egg-case.

Many of the embryos were found to be living till about fifty-four hours after fertilization. When re-examined twelve hours later they were all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found dead. This happened in spite of repeated changes of water and all efforts to keep it at the water temperature of Adyar.

Incidentally I take this opportunity to record the feeding habit of this form which is quite at variance with the observations of previous workers. *Periophthalmus* is known to be predaceous in its habits.[2] On account of the close similarity in external features and habit the prevalent belief is that *Boleophthalmus* also is carnivorous.[3] I have had the opportunity of making a very careful study of the feeding habits of this form in the Adyar backwater which has revealed that it entirely depends for its food on micro-organisms only. The examination of the stomach contents, throughout the year, of a very large number of specimens has definitely proved beyond any doubt that the chief food of the animal consists of microscopic unicellular algæ, mostly diatoms, both littoral and planktonic. Sometimes copepods are also found among these, but they are very rare except in two instances where the stomach contained fairly large quantities of them. The non-predaceous habit of this form is corroborated by the observations made on the living specimens both in the Laboratory aquarium as well as in their natural habitat. In captivity even if the animal has to starve for weeks together it has not been noticed to feed, though a variety of food has been offered.

Petroscirtes bhattacharyæ Chaudhuri.

This form was first described by Chaudhuri[4] in 1916 from Chilka Lake. Two large specimens were captured from Adyar from inside oyster shells, of which one was guarding its eggs. Bhattacharya (1917) described the larvæ of this form belonging to three different stages collected from Chilka Lake. Apart from this nothing is known about its breeding habits and development.

Eggs of *P. bhattacharyæ* were obtained in Januray 1936 when they were found inside an empty oyster shell attached to a stone at a depth of about two and a half feet of water, in front of the Theosophical Society Library, Adyar. There were about 900 eggs most of which were attached to the lower valve adhering to the rock, the upper valve containing only a very small number. The eggs were of a dull orange colour except a few towards the periphery which were beautifully pink. The whole lot was arranged in a compact manner without leaving any space between them (Fig. 61).

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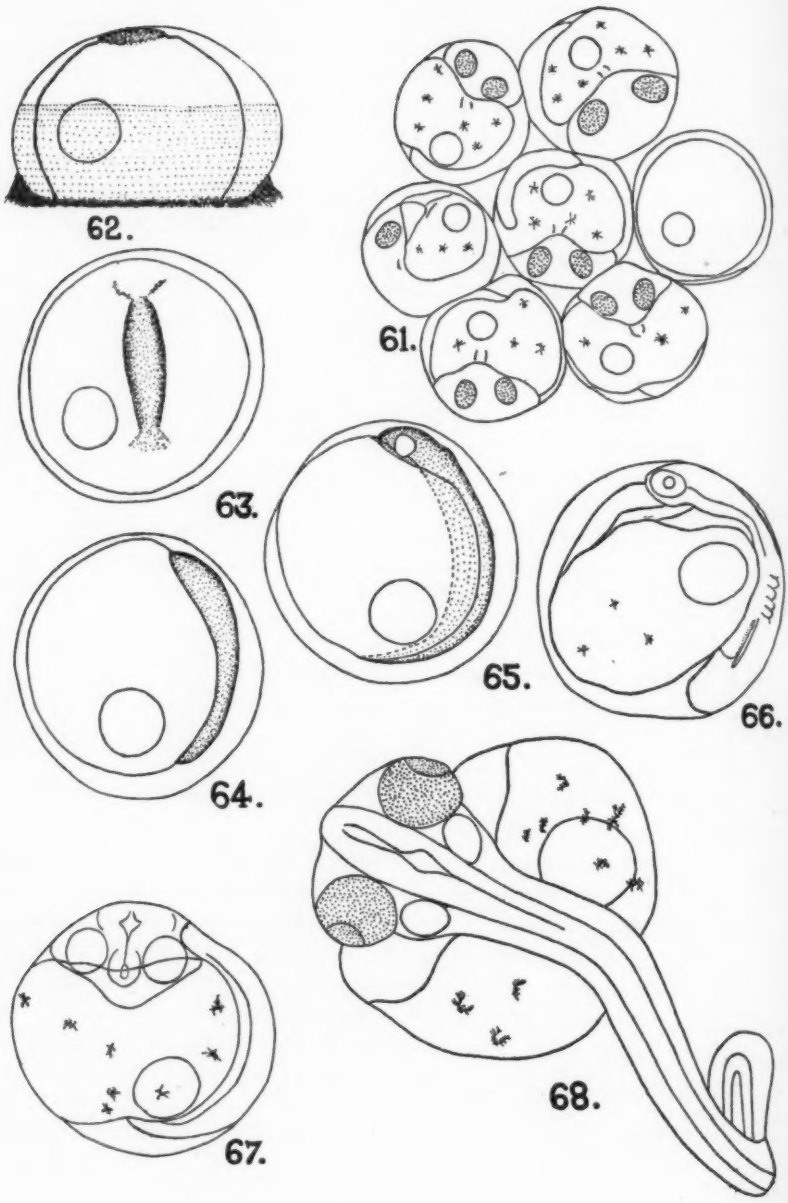
[2] Day, *Fauna of British India, Fishes*, 2 ; Harms, J. W., *Zeit. f. wiss. Zool. Bd.*, 1935, 146.

[3] Alcock. *A Naturalist in the Indian Seas*, London, 1902 ; Jordan, D. S., *Guide to the Study of Fishes*, New York, 1905, 2.

[4] *Records of the Indian Museum*, 1916, 12.


62.
61.
63.
65.
66.
64.
67.
68.

Each egg has the shape of a depressed dome (Fig. 62) with the attached ventral side flat, giving it a circular outline when viewed from above. The height is 0·53 mm. and diameter 0·80 mm. Its peculiar shape should have been brought about only after oviposition. The flat ventral side and the greater part of the curved surface are covered by the adhesive substance which helps in the attachment of the egg.

An interesting feature observed was the considerable disparity in development found among the eggs of the same brood. In a few, towards the periphery of the egg mass only the embryonic ridge was just formed (Fig. 62) and these did not show more than two or at the most three days'-development. These were quite healthy ones and their development was followed. Most of the remaining eggs were in a much advanced state of development, showing a difference of about six days between them. In these the embryos were fully formed with pigmented eyes, complete blood circulation, etc. (Figs. 68 and 69). Intermediate stages between these were also found.

First Day.—Eggs showing very early stages were chosen for following the development. These were the pink ones, situated towards the periphery. The embryonic ridge could be seen as a clear band at the top of the yolk mass (Fig. 63). A single large characteristic oil globule was present in all the eggs including those of the advanced stages.

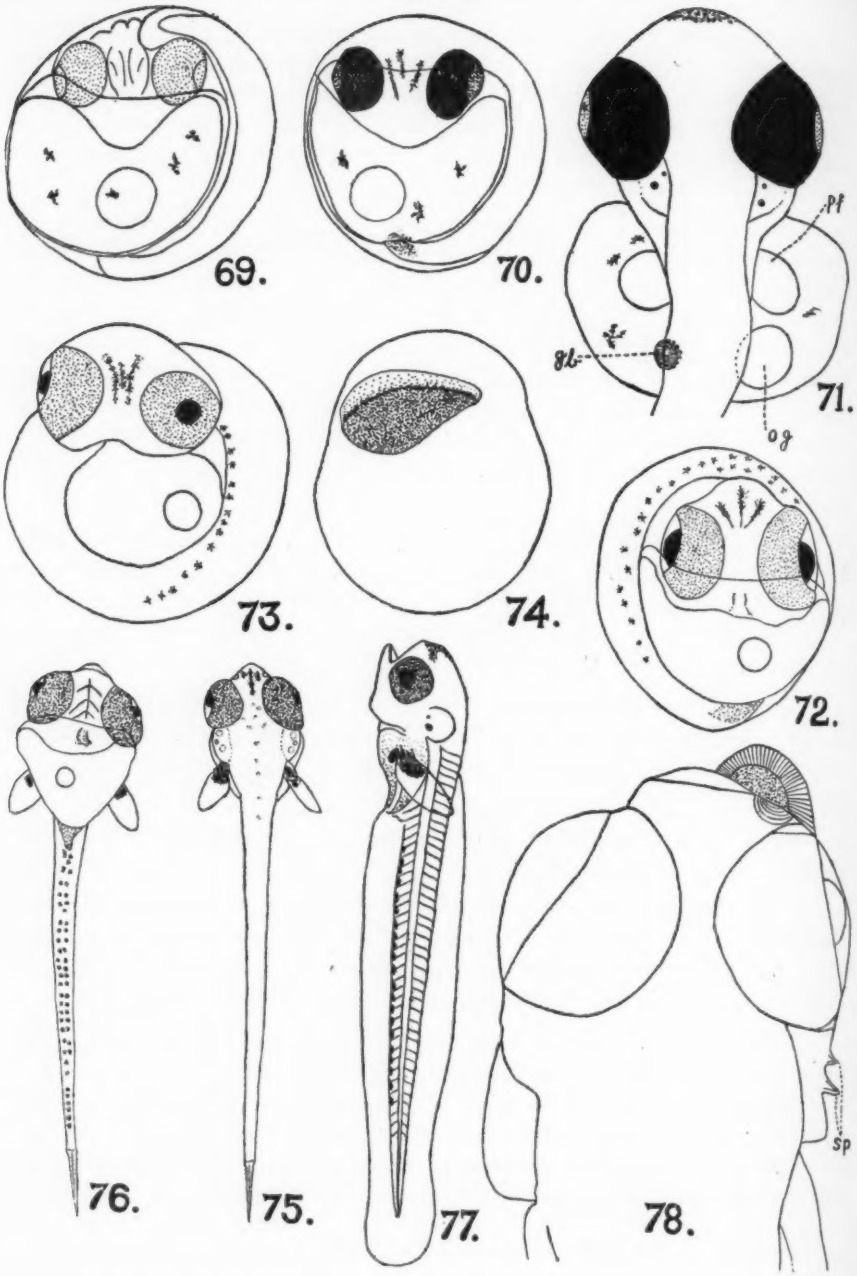
Second Day.—As development proceeds the embryo situated on the dorsal side of the egg gradually turns to one side (Fig. 64). The process is slow as it is considerably handicapped by the peculiar shape of the egg.

Third Day.—The optical vesicles now form (Fig. 65) and the embryo sinks partially into the yolk mass.

Fourth Day.—Some of the somites in the middle of the trunk are now conspicuous (Fig. 66). The caudal end grows free from the egg mass. The heart has begun to beat and a slow circulation is visible. Scattered pigment appears on the yolk mass. The neural groove is distinct.

Fifth Day.—The embryo rolls over and the yolk mass is now on the upper side (Fig. 67). The tail gets elongated and is always kept on one side. The blood circulation is completed. The vitelline circulation is very characteristic as in the European blenny, *Anarrhichas lupus.*[5] Pigmentation increases. The eyes are devoid of any pigment. The beautiful pink colour of the yolk is lost and it changes into dull orange.

[5] McIntosh and Prince, *Trans. Roy. Soc. Edin.*, 1890, **35**, part iii.


69.
70.
Pf
gb
71.
og
73.
74.
72.
76.
75.
77.
78.
SP

Sixth Day.—The lenses are formed. The eyes acquire a dark tinge. The auditory vesicles become visible (Figs. 68 and 69). It was in this condition that the majority of the eggs were found at the time of collection (Fig. 61).

Seventh Day.—Pigment is formed on the inner side of the abdominal wall, giving it a dark appearance, which on casual observation would appear as external. Two minute concretions are formed in the auditory vesicles. Pigment appears on the anterior end of the head above the fore-brain (Fig. 70).

Eighth to Tenth Day.—Pectoral fins are developed (Fig. 71). Gall bladder becomes visible. The yolk gets gradually reduced, as a result of which it loses its original round condition, and becomes first heart-shaped and then crescent-shaped. The oil globule becomes smaller in size. The eyes turn bright and shining.

Eleventh Day.—Yolk is considerably used up as a result of which the orange colour imparted to the embryo is lost. The oil globule still persists, though reduced in size. The pigmentation in front increases and two rows of pigment are formed on the ventral side of the tail portion (Fig. 72).

Twelfth Day.—The larva comes out, head first, by an opening made on the upper side of the shell. At the time of hatching very little yolk remains though the oil globule persists. Fig. 73 shows a larva just in the act of coming out and Fig. 74 an empty egg shell.

It was said at the beginning that the eggs contained both early as well as advanced stages of developing embryos. This difference in the development was kept up till the hatching time, when the first set hatched out seven days before the last. The time taken in the Laboratory for the eggs which showed the earliest stage of development was twelve days and these could not have been more than three days old when collected. So roughly it takes about fifteen days for the eggs to hatch out—a very long time when compared with that of most other fishes at Adyar.

Larva : *First Day.*—A newly hatched larva measures 3 mm. in length (Figs. 75 and 76). From the time of its coming out it begins to move about actively remaining mostly at the bottom. The mouth opening which is absent at the time of hatching is developed on the first day itself. The pectoral fins are fairly large and functional and their bases are darkly pigmented. The dorsal, caudal and anal are continuous. A pre-anal fin is absent. Ventrals are undeveloped. A characteristic feature is the presence of two rows of chromatophores on the ventral side of the body behind the anus (Fig. 77). The pigmentation formed early in front of the head of the

embryo still persists. Apart from this there are tiny scattered pigment granules on the head and body. An opercular spine becomes visible on each side as a minute cone.§

The youngest lot of larvæ of *Petroscirtes bhattacharyæ* described by Bhattacharya (1917) is about 3·25 mm. long. The arrangement of the chromatophores in them is as in the newly hatched one. Those described by him should be some days old as is evident by the greater length of the body and opercular spines.

Larva: *Second Day*.—The lower jaw grows forwards and projects beyond the upper. The opercular spines become prominent and a second pair grow in front. The pectoral fins get more pigmented. The yolk is completely absorbed (Fig. 77).

Larva: *Third and Fourth Day*.—The opercular spines grow longer and appear as in Fig. 78. The larvæ did not live in the Laboratory for more than four days. Bhattacharya mentions only one spine in the youngest lot of larvæ described by him. Hence, it is very likely that only one of the two spines on each side grows long as the embryo grows in size. In the two lots of older larvæ also described by him, the opercular spines are present. The characteristic chromatophores on the ventral side have disappeared and except for the presence of prominent opercular spines many of the adult features have formed. The second and the third sets of larvæ measure 13·25 and 15.9 mm. respectively.

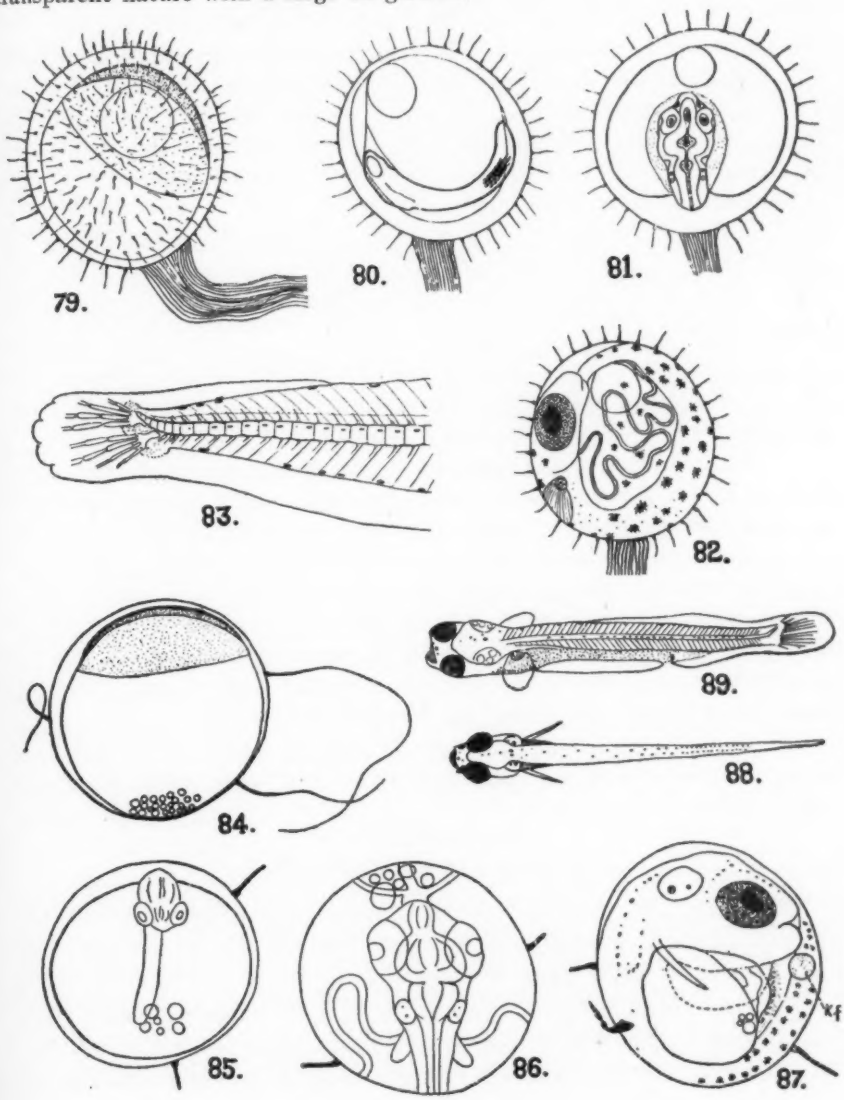
Aplocheilus melastigma McClelland.

Aplocheilus melastigma and *Panchax parvus* are well known as insect larvicides. Both forms are common in the fresh and brackish waters of Madras and their eggs usually found attached to algæ, can be obtained throughout the year from the Cooum and Adyar except in times of very heavy rains. Brief accounts of their development are given below:—

The eggs of *A. melastigma* are generally found attached to algal filaments (*Enteromorpha* and *Chætomorpha*) at Adyar and Cooum either singly or in small clusters. Before the eggs get attached to the algæ they are carried about by the mother in large conspicuous clusters on the ventral side first noticed by Jenkins (1910). Sundara Raj (1916) has given diagrams and descriptions of the eggs and young larvæ, though in the figures of the eggs the threads are shown too stout and sparse. No detailed account of their development is given.

§ The opercular spine, which is only a juvenile feature, is absent in the adult.

Eggs are round and 1·1 mm. in diameter, covered all over with short filamentous threads which are too short to be of an adhesive nature (Fig. 79). From one region alone a thick cluster of long slender threads is given off which helps in the attachment of the egg to the algæ. The yolk is of a clear transparent nature with a large oil globule.



The earliest stage of development that I have observed in the eggs attached to the ventral side of the mother is that in which the blastoderm has formed and has begun to spread round the yolk (Fig. 79). The embryonic region becomes clear on the second day (Fig. 80). The embryo lies sunk in the yolk. The rudiments of the eyes and the neural groove are visible.

On the third day (Fig. 81) the neural groove is large and conspicuous. The embryo becomes larger in size. Auditory vesicles are formed. The eyes begin to get pigmented and the lenses become visible. Circulation has begun, though not very vigorous. The somites are very clear throughout the trunk. The tail gets elongated and freed from the yolk. The embryo is incapable of making any movement.

On the fourth day the eyes are strongly pigmented. The yolk sac circulation is complete. The tail grows much longer and comes round the yolk. Pigment is formed in the body including the yolk sac. The animal wriggles inside the egg membrane.

On the sixth day (Fig. 82) the eyes become bright and shining. The chromatophores get definitely arranged in the body. No appreciable change except in the reduction of the yolk is noticed till it hatches out.

The minimum time for hatching is eight days and by this time practically all the yolk is absorbed. The larva moves about actively as soon as it comes out and measures 4·5 mm. in length on the first day. It always swims along the surface of the water. The dorsal fin which is continuous with the caudal is very small and starts far behind. The caudal fin is heterocercal and provided with fin rays (Fig. 83). The arrangement of the chromatophores in five rows on the trunk, one dorsal, two lateral and two ventral, is very characteristic. The young larva and the changes that take place are described and figured by Sundara Raj (1916).

Incidentally it may be stated that though the majority of eggs hatch out in a fortnight's time, in exceptional cases some eggs had their hatching period unduly prolonged.

Panchax parvus Sundara Raj.

This form was first described by Sundara Raj (1916) from Madras. The animal breeds most intensively about February though the eggs have been collected in other parts of the year also.

Eggs are round and each measures 1·3 mm. in diameter. One to three long threads, sometimes as long as 15 mm. or more are given off from the surface of the egg which help it to get attached to algæ as soon as it is

extruded.[6] The figure of the egg of *Panchax parvus* given by Sundara Raj is not however accurate. The threads from the egg are neither so numerous nor so short as shown by him.

The earliest stage examined is one in which the embryonic ridge has formed and the blastoderm has begun to spread downwards (Fig. 84). The yolk is clear and transparent with a small group of oil globules which persists till the animal hatches out.

By the second day the blastoderm completely envelopes the yolk mass (the neural groove is visible) and rudiments of the eye are formed (Fig. 85). The oil globules coalesce and become larger and fewer in number. The circulation which begins on the third day is complete by the fourth (Fig. 86). The embyro grows in size. The neural groove becomes large and pectoral fins appear. A Kupffer's vesicle becomes visible at the place where the tail end gets free from the yolk mass. Gradually the yolk gets reduced. Pigment is formed in the body, first of a dull brown colour which later turns dark. They get definitely arranged by the seventh day (Fig. 87). The Kupffer's vesicle becomes large and has a beautiful pink colour (Fig. 87 *Kf.*). This is absent in *A. melastigma*. The eyes are black and shining and the head at this time appears proportionately large. Active respiratory movements can be observed and the fluid inside the egg-case is kept in constant motion by the rapid movement of the pectoral fins.

The hatching takes place usually on the eighth day. The larva is 6 mm. long and is extremely agile in its movements from the time of hatching. The arrangement of chromatophores in six rows on the trunk, two dorsal, two lateral and two ventral, is very characteristic of the larva (Figs. 88 and 89). The lower lip is pigmented and projects somewhat beyond the upper. The Kupffer's vesicle disappears on hatching. The dorsal, the caudal and the anal fins are all continuous and the caudal, which is heterocercal, alone is provided with fin rays. The abdominal portion of the animal is considerably longer than that of *A. melastigma*, on account of which the anus is situated far behind. The larva mostly keeps to the surface of the water.

General Observations.

Among the fishes whose breeding habits and development were studied parental care has been noticed in *Etroplus suratensis, E. maculatus, Acentrogobius neilli, A. viridipunctatus* and *Petroscirtes bhattacharyæ*, whereas parental care as such is found to be absent in *Panchax parvus* and *Aplocheilus melastigma*. The fact that in the last-mentioned one eggs are found

[6] Sundara Raj, 1916, attributes to *P. Parvus* the breading habit given by Thomas for *Haplochilus panchax* in his book *Tank Angling in India*, Madras, 1887.

attached to the ventral side of the mother may be interpreted as a sort of parental care though it is clear that the action is not an intentional one on the part of the parent, but brought about by the presence of adhesive threads in the eggs.

The Adyar bar opens at the end of October or by the beginning of November and the river remains connected to the sea till about March-April, though after the end of January the connection is intermittently cut off due to the accumulation of sand. A period of intensive breeding is noted within the brackish water area among fishes during the early pre-monsoon rains at which time the river is not sufficiently flooded to find an outlet into the sea. After the opening of the bar sudden changes in salinity occur in the brackish water zone and breeding is found to be less intensive. Some forms like *Etroplus* go up the river into less saline regions and come back only when the variation in salinity is not sudden. It has been definitely found that *Acentrogobius viridipunctatus*, *A. neilli*, *Panchax parvus* and *Aplocheilus melastigma* are perennial breeders in the brackish water area. Recently (1936) a large number of very young larvæ of *Hemirhamphus limbatus* was found at the end of July and beginning of August after a few showers. Evidently this form breeds about this time.

Acknowledgment.

It gives me very great pleasure to acknowledge my deep indebtedness to Professor R. Gopala Aiyar, Director, University Zoological Research Laboratory, Madras, for his invaluable criticism, constant help and kind encouragement. My thanks are also due to the University of Madras for awarding me a Research Studentship.

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EXPLANATION OF FIGURES.

List of Abbreviations.

1, 2 & 3 Cement Organs ; *eg.*–External Gill ; *gb.*–Gall bladder ; *Kf.*–Kupffer's vesicle ; *og.*–Oil globule ; *pf.*–Pectoral fin ; *sp.*–Opercular spine.

Figs. 1–23. Developmental Stages of *Etroplus maculatus.*

Fig. 1. One celled stage 30 minutes after fertilization × 40
2. Two „ „ 35 „ „ „ × 40
3. Four „ „ 55 „ „ „ × 40
4. Eight „ „ 1 hour and 30 min. „ × 40
5. 2 Hours and 30 minutes old × 40
6. 5 „ „ 30 × 40
7. 9 „ old × 40
8. 12 „ „ × 40
9. 20 „ „ × 40
10. 24 „ „ × 40
11. 28 „ „ × 40
12. 30 „ „ × 40
13. 32 „ „ × 40
14. 38 „ „ × 40
15. 48 „ „ × 40
16. 54 „ „ × 40
17. 72 „ „ × 40
(Larva in the act of coming out).
18. Empty shell × 40
19. Newly hatched larva × 27
20. A group of young larvæ resting at the bottom attached by their mucus threads. × 8
21. Larva Second Day × 27
22. „ Fourth „ × 40
23. „ Sixth „ × 27

Figs. 24–29. Developmental Stages of *Etroplus suratensis.*

24. A well-developed embryo about 60 Hours old × 27
25. A larva coming out of the shell × 27
26. A larva with the egg capsule around. Semi diagrammatic.
27. Newly hatched larva × 27
28. Larva Third Day × 27
29. „ Eigth Day × 20

Figs. 30–49. Developmental Stages of *Acentrogobius viridipunctatus.*

30. Four celled stage 1 Hour and 30 minutes old × 54
31. Eight „ „ 1 „ „ 50 „ „ × 54
32. Sixteen „ „ 2 Hours old × 54
33. Thirty-two „ „ 2 „ „ 15 „ „ × 54

34. 5 Hours old × 54
35. 7 „ „ × 54
36. 10 „ „ × 54
37. 12 „ „ × 54
38. 18 „ „ × 54 (Side View).
39. 24 „ „ × 54 (Dorsal View).
40. 24 „ „ × 54
41. 36 „ „ × 54
42. 48 „ „ × 54
43. 72 „ „ × 54
44. 96 „ „ × 54
45. 120 „ „ × 54
46. 144 „ „ × 54
47. Newly hatched larva with the shrunken external gills behind the lower jaw. × 40
48. Larva Seven Days old. × 40
49. Auditory Organ of a newly hatched larva. × 170
50. „ „ „ „ *Acentrogobius neilli* × 570

FIGS. 51–60. Developmental Stages of *Boleophthalmus boddærti*.

51. Egg immediately before fertilization. × 40
52. One celled stage 10 minutes after fertilization. × 40
53. Two „ „ 30 „ „ „ × 40
54. Four „ „ × 40
55. Eight „ „ × 40
56. Three Hours old × 40
57. Seven „ „ × 40
58. Seventeen Hours old × 40
59. Twenty-four „ „ × 40
60. Forty-eight „ „ × 40

FIGS. 61–78. Developmental Stages of *Petroscirtes bhattacharyæ*.

61. Cluster of eggs showing the compact arrangement. × 40
62. Side view of an egg. × 70
63. Earliest stage observed with the embryonic ridge. × 70
64. Embryo Second Day × 70
65. „ Third „ × 70
66. „ Fourth „ × 70
67. „ Fifth „ × 70
68. „ Sixth „ × 120 (Extracted)
69. „ „ „ × 70
70. „ Seventh „ × 70
71. „ Eighth „ × 120 (Extracted)
72. „ Tenth „ × 70
73. Larva coming out, Twelfth Day × 70
74. Empty Shell × 70
75. Newly hatched larva (Dorsal View) × 40
76. „ „ „ (Ventral View) × 40
77. Larva Second Day (Side View) × 40
78. Head of the larva on the Fourth Day showing the opercular spines. ×170

FIGS. 79–83. Developmental Stages of *Aplocheilus melastigma*.

79. First Day (Earliest stage observed) × 54
80. Second Day × 54
81. Third Day × 54
82. Sixth Day × 54
83. Caudal fin of a newly hatched larva. × 70

FIGS. 84–89. Developmental Stages of *Panchax parvus*.

84. First Day (Earliest stage observed) × 54
85. Second „ × 54
86. Fourth „ × 54
87. Seventh „ × 54
88. Newly hatched larva (Dorsal View) × 20
89. „ „ „ (Side View) × 20

All figures have been reduced to ⅔ the magnifications given.

ON "BIDALOTITE", A NEW ORTHORHOMBIC PYROXENE DERIVED FROM CORDIERITE.

By B. Rama Rao and L. Rama Rao.

Received June 10, 1937.

Abstract.

The paper gives an account of a new type of orthorhombic pyroxene recently discovered in the biotite-cordierite-hypersthene granulites occurring near the village Bidaloti in Mysore. From a detailed study of this mineral from the physical, chemical, and optical points of view, it has been shown that this orthopyroxene has a distinct individuality of its own, distinct from the known members of the enstatite-hypersthene series, and must therefore be recognised as a new mineral. It has also been shown that this pyroxene is of unusual origin, being derived from cordierite. The authors propose to call this new mineral *Bidalotite* after the village near which it was first found.

Introduction.

In the course of a detailed examination of the biotite-cordierite-hypersthene granulites exposed near the village Bidaloti (Koratagere Taluk, Tumkur District, Mysore), an unusual type of a lilac coloured orthopyroxene was noticed by one of us in 1934, and the fact was reported through the columns of *Current Science*.[1] More recently this mineral has been studied in greater detail from the physical, chemical and optical points of view, and the present paper embodies the main results of this investigation.

Geology of the Bidaloti area.

The biotite-cordierite-hypersthene granulite which contains this lilac orthopyroxene is part of a series of crystalline granulitic rocks which occur about half a mile to the south of Bidaloti (13° 31′ N ; 77° 17′ 30″). In the field these granulites may be generally resolved into 3 groups :—(i) biotite-cordierite-hypersthene granulites, and sillimanite quartzites, (ii) pyroxene granulites and (iii) diopside-hornblende granulites and amphibolites. It is believed that the rocks of group (i) represent the metamorphosed products of a thin series of sediments of Dharwarian age,[2] and those of groups (ii) and

[1] B. Rama Rao and M. B. R. Rao, *Curr. Sci.*, 1934, **3**, 114–15.

[2] B. Rama Rao, *Proc. 23rd Ind. Sci. Congress*, 1936, 233.

(iii), the altered phases of basic sills associated with these sediments. This entire composite series is seen to be intruded by the later acid rocks,—the Peninsular gneiss and the Closepet granite.[3] Thus the original sediments of this area appear to have been subjected to two distinct cycles of metamorphism—one due to the intrusion of the basic sills which converted them into a kind of biotite-cordierite hornfels, and the other due to the intrusion of the later granites which brought about a further re-crystallisation resulting in the conversion of the hornfels into a more coarse-grained biotite-cordierite-hypersthene granulite such as we see at the present day. One of the most interesting effects of this latter cycle of metamorphism appears to be the formation in this rock of the lilac orthopyroxene, now under investigation.

The biotite-cordierite-hypersthene granulite.

Striking approximately north and south, this rock occurs as thin bands and shows a dark grey colour when fresh. The texture is variable ; in some places it is very fine-grained and hornfelsic with faint indications of the original banding or lamination, while in other places, especially near the contact with the granite, the rock is quite coarse-grained, and the constituent minerals like biotite, pyroxene, cordierite and quartz could be easily recognised even with the naked eye. Under the microscope, the rock shows a granoblastic to porphyroblastic texture and the following are the minerals commonly seen : cordierite, biotite, hypersthene, quartz, sillimanite, apatite, and the lilac coloured orthopyroxene now under study.

The lilac orthopyroxene.

This mineral is of very common occurrence and is seen practically in all the sections. In some, it occurs as small grains (about 0·1 to 0·3 mm. across) and in others, it is broad and platy being as much as 2 mm. long and 1·5 mm. wide.

A. Optical characters.—The following are the important optical characters of the mineral.

System : orthorhombic.

Habit : granular and platy.

Cleavage : good ; prismatic and at right angles.

Extinction : straight.

Pleochroism : very striking.

Z = lilac, pinkish violet or pale purplish pink.

Y = same as above, but with a slightly brownish tinge.

X = pale yellow to almost colourless.

[3] W. F. Smeeth, *Bull. Dept. of Mines and Geol.*, Mysore State, 1916, 6, 17-18.

Colour absorption : Z = Y > X.

Refractive index : $\alpha = 1 \cdot 656$, $\beta = 1 \cdot 667$, $\gamma = 1 \cdot 672$ } Determined by immersion method.

Birefringence : $\gamma - \alpha = \cdot 015$ (By Bereck's Compensator).

Optical sign : negative.

Optic orientation : Z = C'.

Optic axial angle : 2 V = 57° ± 2° (Determined on Federow stage).

Dispersion : $\rho < \upsilon$ (as determined by the colour fringes of the hyperbola in ordinary white light).

B. Chemical composition.—For purposes of chemical analysis, grains of the mineral were carefully separated by means of Thoulet solution using a Smeeth Separator, every care being taken to see that the sample was as pure as possible. Four separate samples were thus prepared from different specimens and the results of their analyses are as follows :—

Constituents	(1) K/551 (*a*) %	(2) K/551 (*b*) %	(3) K/596 %	(4) K/560 %	(5) G/80 %
SiO_2 ..	53·16	51·36	52·36	52·48	54·37
Al_2O_3 ..	10·55	10·16	4·90	6·76	9·39
Fe_2O_3 ..	4·30	4·43	3·43	5·00	0·95
FeO ..	17·10	17·16	15·43	14·14	18·82
MgO ..	11·95	13·18	18·19	19·61	14·44
CaO ..	..	..	..	..	1·69
Na_2O ..	tr.	..	1·38	..	..
K_2O ..	..	..	tr.	..	..
TiO_2 ..	1·35	0·88	1·73	0·93	0·07
H_2O+ ..	2·06	2·00	1·80	1·00	..
	100·41	99·17	99·22	99·92	99·73
Sp. Gravity ..	3·20	3·24	3·22	3·22	..

(Analyst: Samples 1 to 4—E. R. Tirumalachar, Chemist, Mysore Geological Department.)

In Nos. 1 and 2, the mineral is deep lilac and highly pleochroic.
No. 3 ,, is lighter in colour but still distinctly pleochroic.
No. 4 ,, is pale purplish pink.
No. 5 ,, is an aluminous hypersthene from Uganda included here for purposes of comparison.

From these analyses, it will be seen that this mineral is essentially a metasiiicate of iron and magnesia with some alumina—the amount of alumina, however, being variable. The H_2O in these analyses represents water which is "constitutional" and not "occluded".

C. Relation to cordierite.—Even a cursory examination of the microsections will reveal the fact that the lilac orthopyroxene almost always occurs closely associated with the cordierite of the rock. The grains of cordierite may frequently be seen to pass insensibly into the lilac mineral and in many instances (K/595, K/603)* all gradational stages of alteration from incipient lilac patches in cordierite to well-developed crystals of lilac orthopyroxene with relics of original cordierite are observed (*vide* Pl. XXII, Figs. 1, 2 and 3). In some sections (K/551, K/603 (*a*), K/860) the cordierite shows sector or stellate twinning and portions of one or more of these sectors are often seen to be composed of the lilac mineral (*vide* Pl. XXIII, Fig. 4); in fact cases are not wanting where a stellate twin of cordierite has been entirely replaced by the lilac mineral (K/592, K/603, K/598). The normal cordierite in many of these sections has vivid pleochroic haloes showing the characteristic lemon yellow to orange yellow colour. Similar pleochroic haloes though showing a slightly different shade of colour are also frequently seen in the lilac orthopyroxenes (*vide* Pl. XXII, Figs. 1 and 2). Where we have a cordierite (K/890, K/879) in partial stages of conversion to the lilac orthopyroxene, the pleochroic haloes lying in the cordierite portions are yellow while those in the lilac orthopyroxene are greenish. In addition, we have also a few sections (K/592, K/555, K/860) where one half of a pleochroic halo lies in the cordierite and the other half, in the lilac orthopyroxene. On rotating the stage we find that the former half shows the usual lemon yellow colour while the latter has the greenish tinge elsewhere seen characteristically in the haloes entirely within the orthopyroxenes. From these several evidences it seems obvious that the lilac orthopyroxene is in some way genetically related to the cordierite of the rock.

D. Relation to hypersthene.—Next to cordierite and the lilac orthopyroxene, hypersthene is the most commonly occurring mineral in many

* Numerals in brackets refer to the Reg. No. of specimens and their corresponding microslides kept in the Museum, Mysore Geol. Dept.

of the sections ; and one would naturally like to know what relationship, if any, the lilac orthopyroxene has to this well-known orthopyroxene which occurs with it. From an examination of the sections, it will be seen that these two orthopyroxenes though they occur side by side are always distinct and have nothing to do with each other. In fact the difference in pleochroism and relief between the two minerals are strikingly noticeable (*vide* Pl. XXIV, Figs. 8 and 9) when they occur together (K/548, K/560, K/862). The hypersthene occurs in its usual form and has a relief appreciably higher (Ref. Index = 1·72) than the lilac mineral (Ref. Index = 1·67). It always shows its characteristic pleochroism (X = bright pinkish red, Z = bluish green) which comes nowhere near that of the lilac orthopyroxene (X = pale yellow to colourless, Z = lilac or purplish) ; and the nature of the dispersion is also different in these two minerals. Thus it is clear that the lilac orthopyroxene is a distinctly separate mineral, with no relationship whatever in its optical characters to hypersthene. Moreover, in none of the hypersthenes of the rock do we see any pleochroic haloes such as those found in the cordierite and the lilac orthopyroxene.

Discussion.

From the above description, it will be evident that the lilac mineral under investigation has a distinct individuality of its own, and represents a new type of orthopyroxene, different from any of the usual members of the enstatite-hypersthene series. The intimate association between this new pyroxene and cordierite to which attention has been drawn above is such that it leads one naturally to conclude that the former is in some way derived from the latter. This mineral transformation was probably one of the effects of the later granitic intrusions on the pre-existing biotite-cordierite hornfels of the area, and consisted in the chemical breaking up of some of the cordierites, following which there was the elimination of a certain amount of alumina and addition of some ferrous iron and magnesia, and the material thus derived was recrystallised to form the new lilac orthopyroxene. Some of the needles of sillimanite crystals commonly seen in association with the new pyroxene probably represent the products of crystallisation of the alumina liberated in the above process. This lilac pyroxene being essentially a meta-silicate of iron and magnesia is similar in composition to hypersthene, but differs from it in having a certain amount of alumina in addition. Thus at first sight the new mineral might appear to be merely an aluminous hypersthene of the kind mentioned by Groves[4] (see table of analyses) but a look at its optical properties and a comparison with those of hypersthene will at once

[4] A. W. Groves, *Q. J. G. S.*, 1935, **91**, Pt. 2, 155-57.

make it clear that this lilac orthopyroxene cannot by any means be considered merely as a variety of hypersthene. The aluminous hypersthenes described by Groves in spite of the alumina they contain, are like normal hypersthenes in their optical characters, but such is not the condition in the present case. Here we have a new mineral altogether.

Conclusion.

Thus in this Bidaloti area we seem to be dealing with an entirely new type of orthopyroxene and that of an unusual origin, derived from cordierite. In its mode of origin, optical characters, and to a certain extent, its chemical composition, this Bidaloti lilac mineral has an identity of its own and has therefore to be recognised as a new member of the orthopyroxene group. This new mineral may appropriately be called "Bidalotite" after the name of the village near which it was first found.

Acknowledgments.

The authors desire to acknowledge their indebtedness to Sir C. V. Raman, Director, Indian Institute of Science, Bangalore, for permission to use the Federow stage of the Institute Laboratory; to Messrs. T. P. Krishnachar and M. B. Ramachandra Rao of the Mysore Geological Department, for occasional help in the field and laboratory; to Mr. E. R. Tirumalachar of the same Department for the chemical analyses and to Mr. K. Shankar Rao, for the drawings of some of the microsections.

EXPLANATION OF PLATES.

Plate XXII.

Fig. 1.—An early stage of development of the lilac orthopyroxene. In the centre of the figure the mineral is seen forming a closely packed fibrous aggregate in the original cordierite. The unaltered cordierite can still be seen at the edges. (K/606.) × 40.

Fig. 2.—In this slide the mineral has developed a pronounced lilac tinge, and also shows a slightly higher relief than the grains of cordierite. Note the pleochroic haloes both in the cordierite grains (*c*) and the newly-formed lilac mineral (*l*). (K/595.) × 40.

Fig. 3.—The lilac orthopyroxene is seen here as a clearly recognisable mineral, coarse and platy and showing good cleavages. Remnant patches of cordierite (*c*) are still noticeable. Note the sector twinned cordierite, clear and unaltered, at the lower end of the figure. (K/603 *a*.) × 24.

Plate XXIII.

Fig. 4.—In the centre of the figure is seen a sector twin of cordierite portions of which are composed of the lilac mineral. (K/551.) × 24.

Fig. 5.—Coarse grain of a twinned cordierite crystal converted in portions to the lilac mineral. Needle like crystals in cordierite (colourless) are sillimanite. (K/860.) × 60.

FIG. 6.—Sieve texture in the lilac orthopyroxene. The irregular granular inclusions of the colourless minerals are composed of mostly quartz and some cordierite. (K/551.) × 30.

PLATE XXIV.

FIG. 7.—The figure shows a very coarse crystal of the lilac orthopyroxene with its well-developed prismatic cleavages. Note the irregular and sutured boundary, at the lower end of the figure between the lilac mineral and cordierite. (K/871.) × 20.

FIG. 8.—In the centre of the figure is a well-developed platy crystal of the lilac mineral surrounded by granular crystals (dark in figure) of hypersthene. Note the contrast in relief between the two minerals. (K/862.) × 20.

FIG. 9.—Figure shows the lilac mineral and hypersthene together. Note the difference in their colour absorption for similar orientations and also the contrast in relief between the two minerals. (K/560.) × 20.

FIG. 1.

FIG. 2.

FIG. 3.

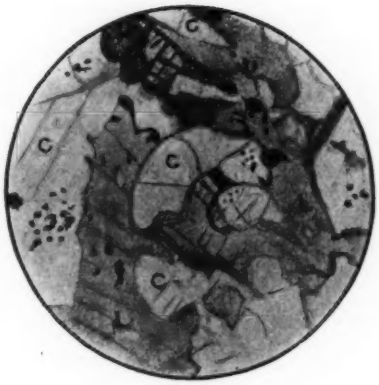

FIG. 4.

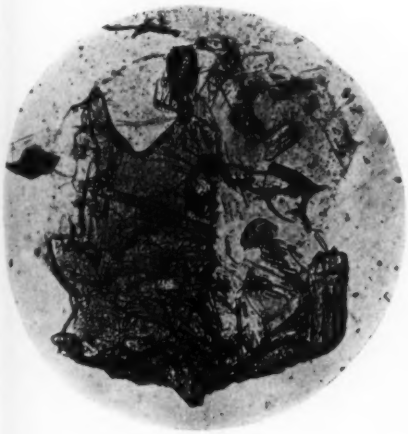

FIG. 5.

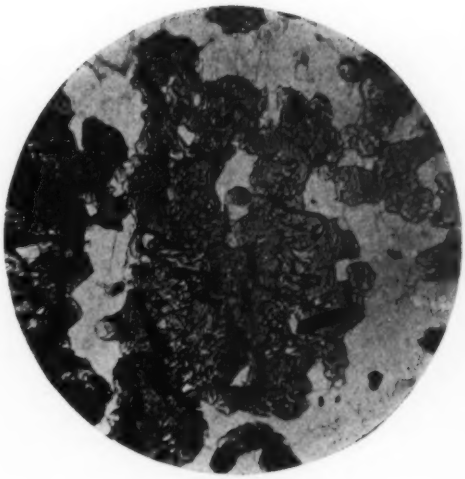

FIG. 6.

FIG. 7.

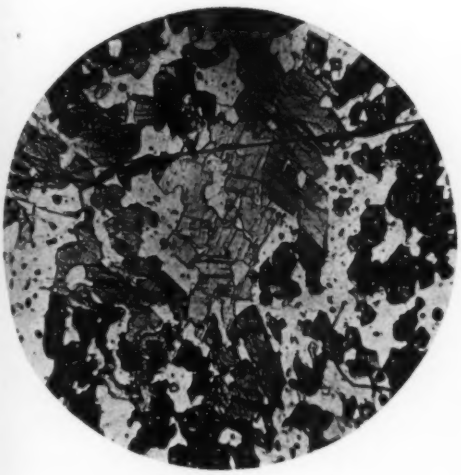

FIG. 8.

FIG. 9.

GENUS *ZYGNEMOPSIS* IN NORTHERN INDIA.

By M. S. Randhawa, M.Sc., I.C.S.
(Fyzabad.)

Received April 17, 1937.

In 1934, the author described a new member of the Conjugatæ *Ghosella indica*, as a new genus and a new species. In subsequent years and particularly in 1937, he came across five more species which may be referred to this new genus.

Before dealing with these new species, which form the subject-matter of this paper, it is advisable to review the history of this new genus which occupies a unique position in the order Zygnemales due to its transitional nature. That these algæ stand midway between *Debarya* and *Zygnema* there is no doubt. If one were to consider the character of their chloroplasts only, then there is nothing to differentiate these from the other species of *Zygnema*. In fact in a purely vegetative stage, nobody can distinguish these algæ from some species of *Zygnema*. In their reproductive phase these algæ show a considerable resemblance with some species of *Debarya* with certain peculiarities of their own. Mucilaginous lamellæ of pectic cellulose, very wide conjugation canals, marked geniculation of conjugating filaments, and isogamous conjugation with the zygospores extending into the gametangia are the features which these algæ have in common with *Debarya*. Due to its resemblance with *Debarya*, Transeau in 1915 described certain species which are now referable to this genus as *Debarya americana* Trans. and *Debarya decussata* Trans. In 1925 he described *Debarya spirale* (Fritsch) Trans. Czurda who bases his classification of Zygnemales on the character of the chloroplasts only, in Heft 9 of *Susswasserflora Mitteleuropas* described all these three forms as species of *Zygnema*.

However, apart from the resemblance of these forms in their vegetative phase with *Zygnema*, and in their conjugation with *Debarya*, they have certain peculiarities of their own. Firstly, the zygospores of these algæ show a great variety in shape and structure even in the same species. Secondly, all these are characterised by the presence of peculiar triangular zygospores produced as a result of the fusion of the terminal cell of a filament with the intercalary cell of another filament. Thirdly, in the presence of spindle-shaped or oval aplanospores these algæ differ from most known species of *Debarya*.

Moreover, if these algæ are placed in the genera *Zygnema*, and *Debarya* according to the whim of the taxonomist, it will cause a great confusion. Their transitional nature is evident by the combination of the features of *Zygnema* and *Debarya* which they show, and they mark a definite stage in the evolution of the Zygnemales. So the best course is to include all these in a new genus which the author named in 1934 as *Ghosella* and Transeau in the same year as *Zygnemopsis*. In the present paper the author has adopted the generic name suggested by Transeau, for it is more descriptive of the nature of the algæ described under it. So far in all fourteen species have been described from Europe, South Africa, America and China, as belonging to this genus. With the addition of the six Indian species the total stands at twenty.

Genus *Zygnemopsis* is described as follows :—

Zygnemopsis (Skuja) Transeau 1934 = *Ghosella*. Randhawa 1934.

Vegetative cells with plane end walls ; two more or less rounded or semistellate chloroplasts containing a central pyrenoid, in each cell. In some rare cases one or three to five chloroplasts may be seen in each cell. Reproduction takes place by isogamously produced zygospores, and spindle-shaped aplanospores. No lateral conjugation takes place in any known species. Marked geniculation of the mating filaments takes place during conjugation. The gametangia become filled with a dense pectic cellulose colloid, which in most species is secreted in lamellæ, and in a few in a homogeneous mass. The spore wall is composed of three layers, of which the mesospore is yellowish to brownish in colour and is variously sculptured at maturity. The zygospores show a great variety in shape, even in the same species. The remains of the gametangia persist around the zygospores in the form of horn-like structures. Peculiar triangular zygospores produced as a result of the fusion of the terminal cell of a filament with the intercalary cell of another are also seen.

1. Zygnemopsis indica Randhawa (Fig. I).

Vegetative cells 10–15 μ broad, 4–5 times as long, each with two rounded or semistellate chloroplasts, with one pyrenoid in each, surrounding the centrally situated nucleus (Fig. I, 1).

Conjugation.—Conjugation scalariform, distinct geniculation of filaments takes place. Conjugation canals very wide (Fig. 1, 2). Deposition of shining white lamellæ of pectic cellulose takes place in the gametangia. Conjugation between 5 or more filaments quite common.

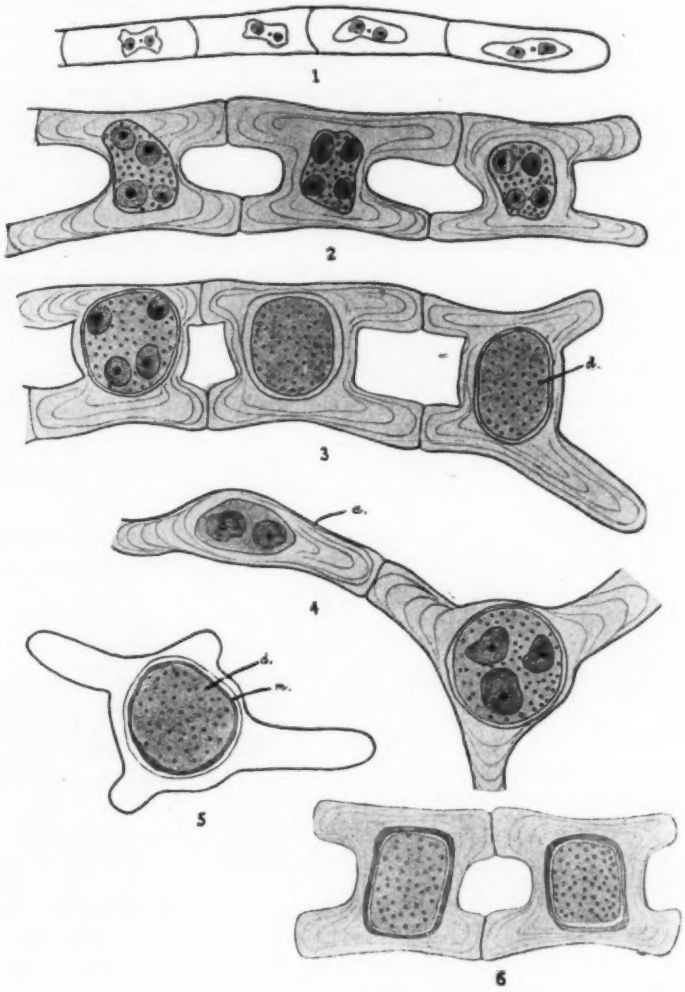

FIG. 1. *Zygnemopsis indica* Randhawa.

1. A vegetative filament showing cells and chloroplasts. 2. A stage in conjugation showing wide conjugation canals and lamellation. 3. Two conjugating filaments showing ripe zygospores. 4. A filament showing a triangular zygospore and an aplanospore (*a*). 5. A ripe zygospore showing the spore walls, mesospore (*m*) shows punctation. Lamellæ are not shown in the walls. 6. Shows quadrately ovoid zygospores.

All figs. × 660.

Zygospores in the conjugation canal, also extending into the gametangia The joints of the ripened cells get loosened, resulting in the detachment of the zygospores which retain the four horn-like remains of the gametangia. The zygospores are 36-46 μ in diameter exclusive of their mucilaginous coats, and including these may be as broad as 56 μ. When fully mature these are deep yellow in colour, and show a great variety in shape, some being quadrately ovoid (Fig. I, 6), some irregular but the majority are of the globose type. The zygospore wall is composed of three layers of which the exospore is thin, smooth, light blue, the mesospore is thick, chocolate brown, and the endospore is yellowish brown. The zygospore wall shows punctation on its surface in the form of very small circular depressions (Fig. I, 3, 5 and 6).

Peculiar zygospores with three horn-like outgrowths, resulting from the conjugation of a terminal cell of a filament with an intercalary cell of another, are also seen (Fig. I, 4).

Aplanospores.—Spindle-shaped aplanospores are also commonly seen (Fig. I, 4).

Habit.—Free floating in a yellowish mass in a fresh-water pond near V. Hamira, district Jullundar, and V. Shahpur, district Hoshiarpur, Punjab, in the months of February, March and April 1930.

2. *Zygnemopsis splendens* sp. nov. (Fig. II).

This alga has been collected mixed with a species of *Mougeotia* from various ponds and paddy fields in villages Mhow-Jadubanspur and Rampur, district Fyzabad, in the months of January, February and early March 1937.

Vegetative cells 12–14 μ broad, 30–42 μ long, each containing two irregularly rounded chloroplasts, each with a central pyrenoid. The protoplasm of the cells secretes a shining white pectic cellulose substance. Septa of the cells plane (Fig. II, 1).

Reproduction.—Scalariform conjugation is the usual mode of reproduction. As compared with the other species of genus *Zygnemopsis*, the conjugation canals are comparatively narrow (Fig. II, 2). The protoplasm secretes a shining white pectic cellulose substance which fills the gametangia in a homogeneous mass, unlike the other species of *Zygnemopsis*, where it is secreted in layers. This is a typically isogamous species and zygospores are found more or less in the middle, and extend into the gametangia only very slightly in immature condition. The zygospores are of various shapes, being heart-shaped, diamond-shaped, or potato-shaped in appearance (Fig. II, 3 and 4). The remains of the gametangia persist around the zygospores, which dissociate from each other by the loosening of the joints

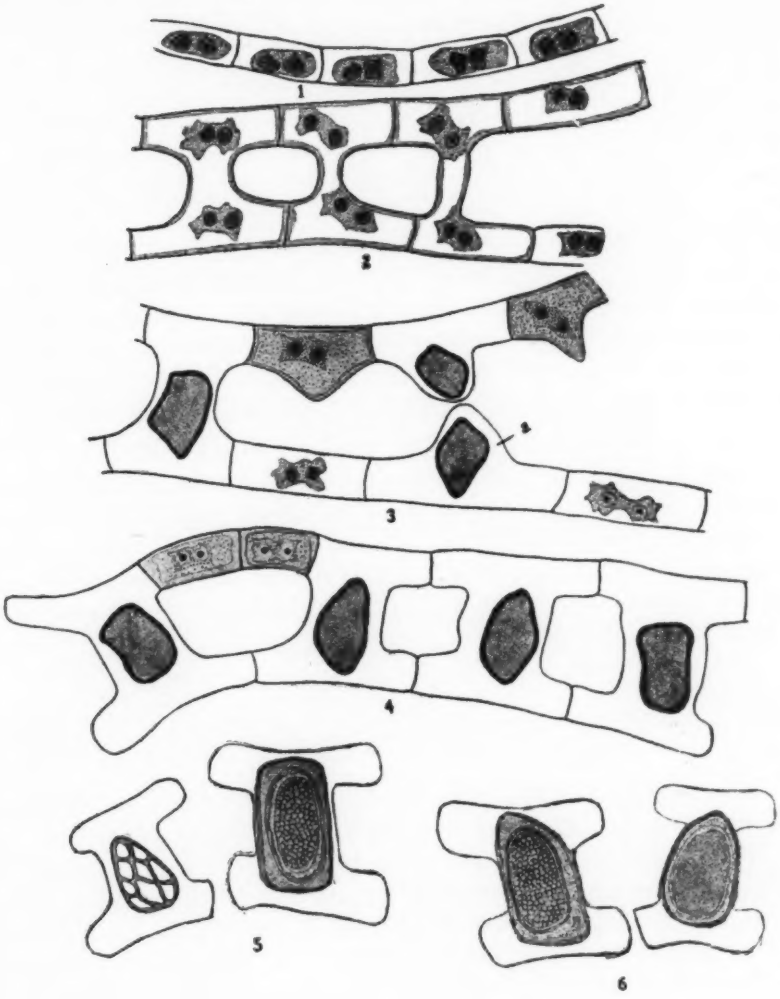

FIG. II. *Zygnemopsis splendens* sp. nov.

1. A filament showing chloroplasts and cells. 2. An early stage in conjugation showing narrow conjugation canals. 3. Two conjugating filaments showing azygospores (*a*). 4. An advanced stage in conjugation showing the shapes of zygospores and absence of lamellation. 5 and 6. Show dissociation of ripe zygospores with the remains of gametangia around them. All figs. × 660.

(Fig. II, 5). The immature zygospores are 16–20 μ long, exclusive of the remains of gametangia. The spore wall bears reticulations on its surface which are faintly brown in colour in immature zygospores, (Fig. II, 5). Mature zygospores were collected on 25th March 1937. These are quadrately ovoid and in some cases more or less conical in shape. As compared with the immature zygospores these are considerably bigger in size being 26–30 μ broad, and 40–50 μ long. The exospore is thin, smooth, light blue in colour and is separated by a wide space filled with brownish yellow matter from the brownish mesospore which bears minute reticulations on it (Fig. II, 6).

Azygospores.—In some cases it was noticed that the gametes failed to meet, and their contents independently develop into azygospores (Fig. II, 3).

Affinities.—This species differs from all the other known species of *Zygnemopsis*, in the secretion of pectic cellulose in a homogeneous mass, in the absence of lamellation and the peculiar shape of its zygospores.

3. *Zygnemopsis lamellata* sp. nov. (Figs. III and IV).

This beautiful member of the Zygnemales was found free-floating in a darkish purple mass in Tons Nadi under the bridge near the tahsil buildings at Akbarpur, district Fyzabad, U.P., on 15th February 1937. Another specimen was collected on 25th March 1937 from Tirwa Nadi, near Tanda. This alga shows a luxuriant growth in the slowly flowing fresh-water streams of Fyzabad District.

The vegetative cells are 15–18 μ broad and 32–42 μ long. There are two rounded or more or less semistellate chloroplasts in each cell. The periphery of the cells are full of pectic cellulose which is shining white in appearance.

Reproduction.—Reproduction in this species takes place by means of aplanospores and zygospores produced by isogamous conjugation.

(*i*) *Conjugation.*—The process of conjugation in this species is typically isogamous. The conjugation canals are immensely wide. The chloroplasts show a movement towards the middle part of the conjugation canal, as is evident from their fore and aft position. Surrounding the chloroplasts are numerous granules. Mucilaginous lamellæ composed of shining white pectic cellulose are secreted by the retreating protoplasm as in *Zygnemopsis indica* (Fig. III, 2). The conjugating filaments show a marked geniculation, while the cell contents become very much vacuolated (Fig. III, 3).

The zygospores are of a dark bluish green colour and occur in such large numbers that they give a dark bluish colour to the water of rivulet from where the alga was collected. They present a great variety in shape, in some cases they are quadrangular with plane sides, and in some cases these

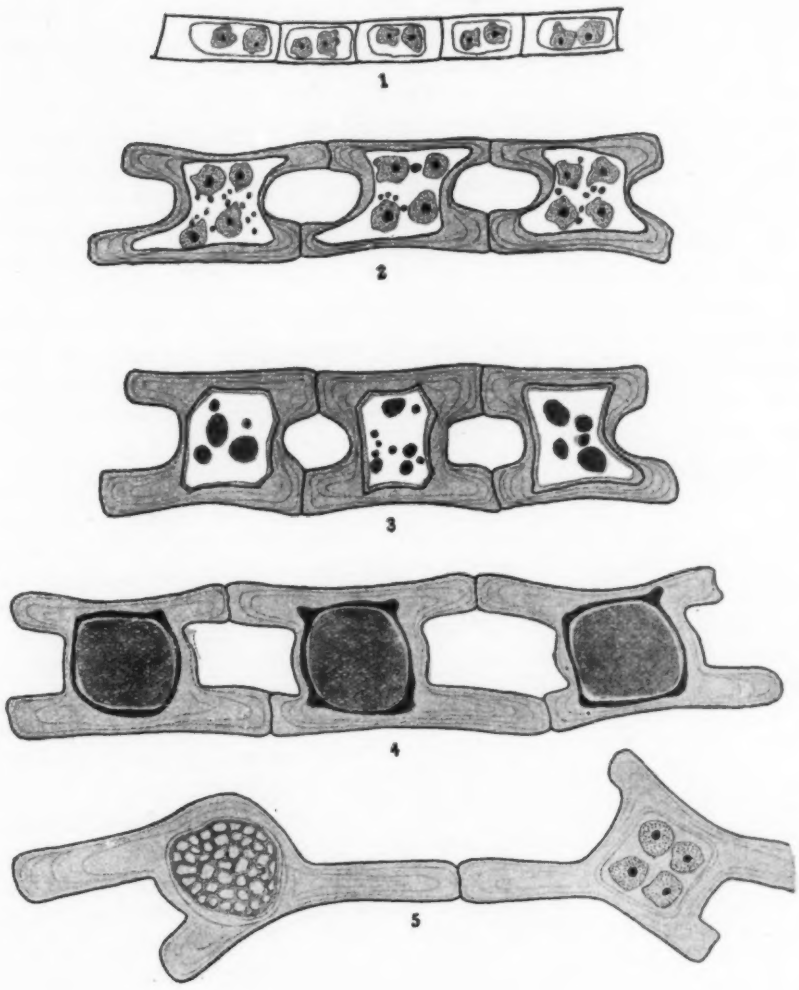

FIG. III. *Zygnemopsis lamellata* sp. nov.

1. Shows a filament with cells and chloroplasts. 2 and 3. Show early stages in conjugation. 4 and 5. S how different shapes of zygospores with their markings.

All figs. × 660.

are quadrangular with concave sides, and the corners obtuse (Fig. III, 4). In some rare cases the zygospores were diamond-shaped (Fig. IV, 2), but on the whole the globose shape is more common (Fig. IV, 1). The zygospores are 44–52 μ in diameter. The spore wall is composed of three layers, of which the exospore is also fairly thick and greenish blue in colour, the mesospore is dark bluish green in colour, and the endospore is thin, light bluish in colour. The spore wall bears foveolate type of reticulations on its surface, and when crushed, punctation in the form of small dots is also noticeable on the surface of the spores (Fig. IV, 1). The zygospores get loosened from each other, and float about with four horn-like remains of the gametangia at their sides.

A peculiar type of triangular zygospores seen in *Z. indica* are also seen in this species. These result from the conjugation of the end cells of some filaments which meet vertically the cells of a filament lying horizontally. The result is the formation of these triangular zygospores with three horn-like sides (Fig. IV, 3).

(*ii*) *Aplanospores.*—Unlike other species of *Zygnemopsis*, reproduction by means of aplanospores is just as common as that by the aid of zygospores. Such aplanospores were also seen in *Z. indica*, but in that species, these are rather rare. In some cases it was seen that while normal conjugation is taking place in a part of two filaments, the remaining part of one is producing aplanospores. In early stages the middle part of a cell begins to expand laterally, while the chloroplasts become surrounded by granular matter. The cells become filled with a solid mass of pectic cellulose at the sides, while around the chloroplasts a reticulum is secreted, which is irregular in earlier stages (Fig. IV, 4). Later on the network secreted in each chloroplast expands, and both the networks meet in the middle forming a cylinder (Fig. IV, 5). A thick, brown and smooth exospore spindle-shaped in outline separated from the reticulate mesospore by a wide distance develops in mature spores (Fig. IV, 7 and 8). However, some aplanospores are typically oval in shape. The mesospore is thick brown with foveolate reticulations. The aplanospores are 24–36 μ broad, and 40–74 μ long, inclusive of the mucilaginous walls. Excluding the mucilaginous sheath these are 18-24 μ broad.

Affinities.— This alga differs from *Zygnemopsis indica* Randhawa in the presence of foveolate reticulations on the surface of spore walls, the peculiar structure of its aplanospores, and the broader size of its cells and zygospores. It differs from *Z. quadrata.* Jao in the white colour of its lamellæ, reticulations on its spore walls, which are scrobiculate in the Chinese species, and the absence of a suture. There is a slight resemblance between

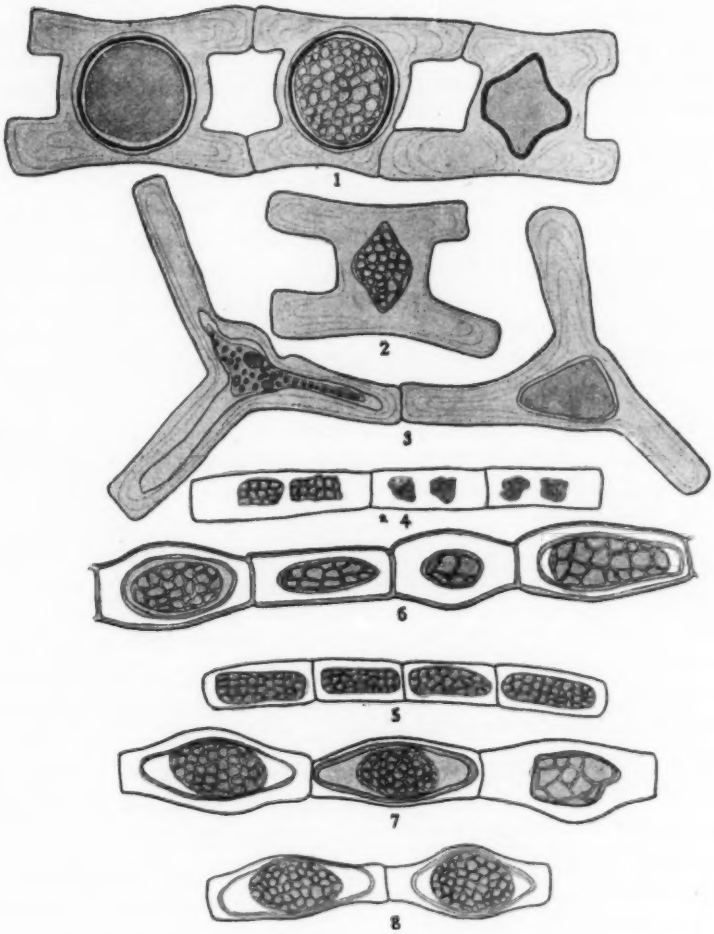

FIG. IV. *Zygnemopsis lamellata* sp. nov.

1 and 2. Show different type of zygospores. 3. Shows a triangular zygospore. 4, 5 and 6. Show different stages in the development of aplanospores. 7 and 8. Show ripe aplanospores.

All figs. × 660.

the aplanospores of this form with those of *Debarya reticulata* Transeau 1925, whose zygospores are not known, but those of the present form differ in the absence of thickenings at the poles, as well as the presence of well-marked foveolate reticulations. All these differences from the related species, warrant the establishment of a new species which is called *Zygnemopsis lamellata.*

Zygnemopsis lamellata sp. nov. (Figs. III and IV).

Vegetative cells 15–18 μ long. 32–42 μ broad, each with two semistellate chloroplasts bearing a pyrenoid in the middle. Reproduction by means of globose or quadrangular zygospores 44–52 μ in diameter, dark bluish green in colour, extending into the gametangia. Lamellæ of pectic cellulose secreted in the gametangia. Mesospore thick, dark bluish green with foveolate reticulations. Spindle-shaped or oval aplanospore also abundantly seen.

Habit.—Found in a dark purple mass in Tons Nadi near Akbarpur, district Fyzabad, U.P., on 15th February 1937 and on 25th March 1937 from Tirwa Nadi, Tahsil Tanda, in the same district.

4. Zygnemopsis Iyengari Randhawa nov. comb.

(*Zygnema Iyengari* Randhawa) (Figs. V and VI).

This species of *Zygnemopsis* was found free-floating in a yellowish brown mass in small puddles of water around Pikia Nadi near Rajeh Sultanpur, Tahsil Tanda, district Fyzabad, U.P., mixed with *Mougeotia lepus* sp. nov. and numerous species of desmids.

The vegetative cells are 12–16 μ broad and 5–8 times as long, being narrower and longer as compared with those of *Z. lamellata.* There are two more or less rounded chl roplasts in each cell (Fig. V, 1).

Conjugation.—The early stages in conjugation are very similar with those of *Z. indica* and *Z. lamellata,* and in the absence of mature spores with their characteristic markings it is impossible to distinguish between the various species. The conjugation canals are wide, well-marked lamellæ of pectic cellulose appear, and the conjugating filaments show geniculations (Fig. V, 2 and 3). When stained with iodine the chloroplasts with their central pyrenoids show a wheel-like structure with a number of spokes radiating outwards (Fig. V, 3, 4 and 5). The arms of the gametangia become filled with pectic cellulose resulting in the confinement of the protoplasm and the cloroplasts in a squarish space in the middle of the conjugation canals (Fig. V, 4). Later on the outer part may become hexagonal in outline,

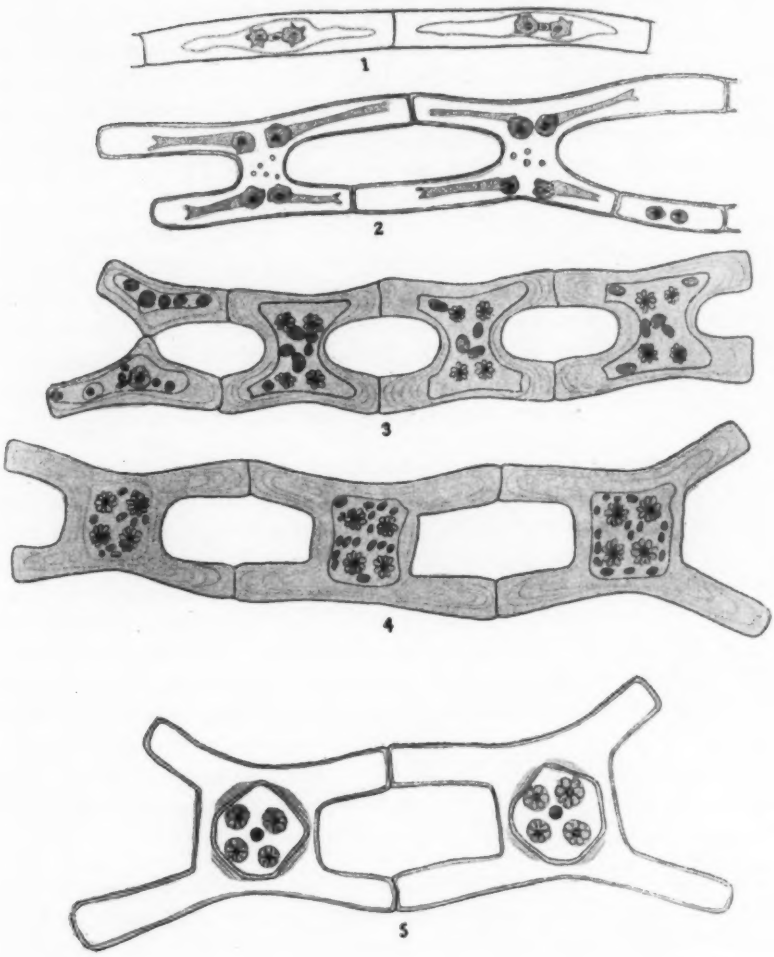

FIG. V. *Zygnemopsis Iyengari* sp. nov.

1. Shows a vegetative filament. 2, 3, 4 and 5. Show different stages in conjugation.

All figs. × 660.

and lamellation becomes very much faint, being noticeable only around the periphery of the central mass of protoplasm (Fig. V, 5).

When fully mature the zygospores completely fill the conjugation canals, and not a trace of pectic cellulose being seen laterally. The spore wall is composed of two layers, of which the exospore is light bluish green with a brownish tinge. In some zygospores the exospore is as thick as the mesospore and shows thickenings at the four poles (Fig. VI, 1). The mesospore is separated from the exospore by a space filled with a yellowish brown matter. In mature spores it is thicker than the exospore, and is yellowish brown in colour. The mesospore is very complexly sculptured, being raised into a number of ridges which are more noticeable in a lateral view (Fig. VI, 3). Denticulate markings in the form of small spine-like projections are also noticeable, and there may also be wave-like reticulate markings on some of the zygospores (Fig. VI, 2). Triangular zygospores are also frequently seen (Fig. VI, 5). Mature zygospores are 44–54 μ in diameter.

Aplanospores.—Asexual reproduction by means of barrel-shaped, spindle-shaped, or globose aplanospores takes place quite commonly in this species. While some filaments may be reproducing exclusively in an asexual manner with plenty of aplanospore formations, others show conjugation side by side with aplanospore formation (Fig. VI, 1). The aplanospores develop from the ordinary vegetative cells by swelling of the middle part and appearance of granular matter around the chloroplasts (Fig. VI, 6). Extensive secretion of pectic cellulose in a homogeneous mass takes place and a reticulum develops in the protoplasm (Fig. VI, 7). When mature the aplanospores are globose or spindle-shaped with a number of parallel ridges on the surface (Fig. VI, 9, 10, 11 and 12). The aplanospores are 24–28 μ in diameter normally, while in one case these were as broad as 32 μ and 54 μ long.

Affinities.—In september 1936, the author described[4] a new species of *Zygnema* as *Z. Iyengari*. That aplanosporic species resembles the present one in the shape of its aplanospores, the only difference being that the cells and aplanospores of the present form are longer and narrower. *Zygnema Iyengari* is obviously an aplanosporic species of *Zygnemopsis* and is renamed as *Zygnemopsis Iyengari*. *Z. Iyengari* differs from the related species *Z. indica* in the presence of ridges on its zygospores and the peculiar shape and structure of its aplanospores.

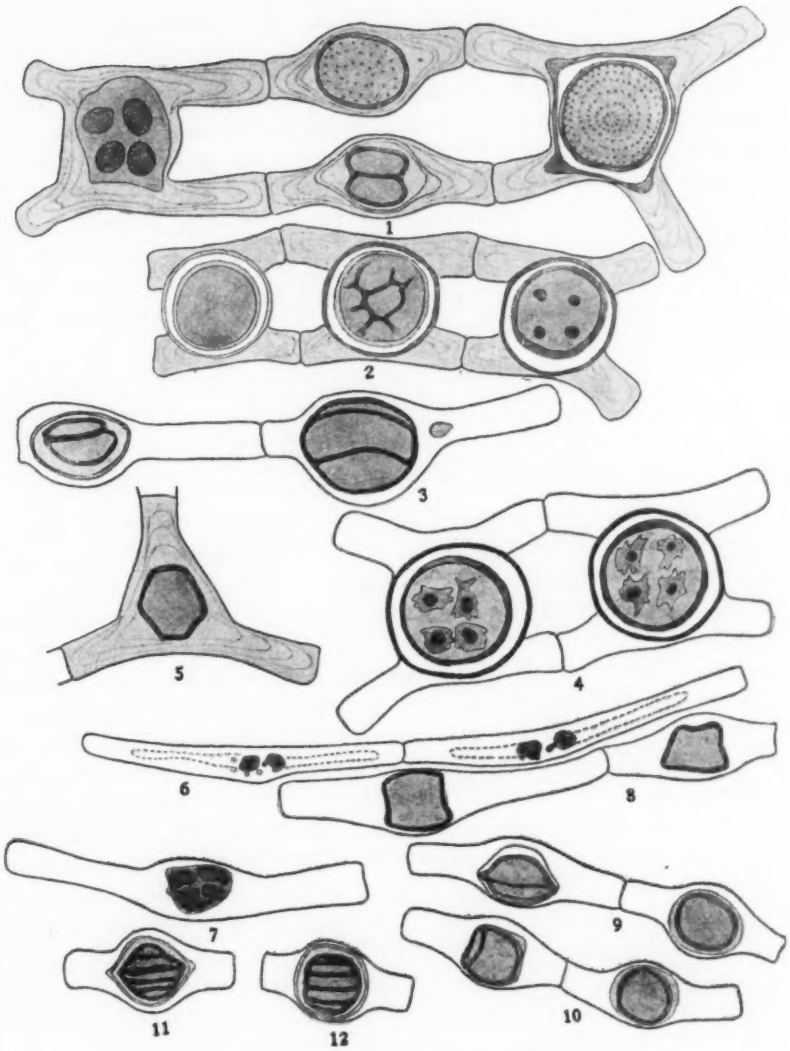

FIG. VI. *Zygnemopsis Iyengari* sp. nov.

1. Shows conjugation and asexual reproduction by aplanospores (*a*) at the same time. 2. Shows ripe zygospore. Mark the reticulate markings on the middle one. 3. Shows lateral view of two zygospores showing ridges. 4. Shows ripe zygospores. 5. Shows azygospores resulting from the fusion of the terminal cell with an intercalary cell. 6 and 7. Show early stages in the development of aplanospores. 8 to 12. Show different types of aplanospores with their markings.

All figs. × 660.

Zygnemopsis Iyengari nov. comb. (*Zygnema Iyengari*). (Figs. V and VI). Randhawa.

Vegetative cells 12–16 μ broad, 5–8 μ times as long, each cell with two rounded chloroplasts bearing each a central pyrenoid. Zygospores globose, 44–54 μ in diameter, with a thick bluish green exospore separated by a space filled with light yellowish brown matter from the yellowish brown mesospore. Spore wall with ridges and denticulation on surface. Asexual reproduction by barrel-like, or spindle-like aplanospores bearing a number of parallel ridges on surface, 24–28 μ in diameter.

Habit.—Free-floating in Pikia Nadi, near Rajeh Sultanpur, district Fyzabad, U.P., on 15th January, 1937.

5. Zygnemopsis globosum sp. nov. (Fig. VII).

This species of *Zygnemopsis* was found free-floating in an irrigation channel leading to paddy fields from a Jhil near Tanda on 25th March, in a dirty brown mass mixed with a species of *Chara* and *Oedogonium*.

The vegetative cells are 12–14 μ broad and 36–72 μ long, and each cell usually contains two rounded chloroplasts, but in some cells there is only one chloroplast in each cell (Fig. VII, 1).

Conjugation is typically isogamous as in other species of *Zygnemopsis* and plenty of mucilage secretion in the forms of lamellæ takes place. Conjugation between three or more filaments is quite common. Conjugation canals are very wide and marked geniculation of filaments takes place (Fig. VII, 3, 4 and 5). Ripe zygospores are yellowish in colour. The outermost covering in most cases is formed of two thick layers of shining white pectic cellulose, beneath which are noticeable the two layers of the spore wall. The exospore is dark yellowish brown in colour and separating it from the mesospore is a space filled with brownish matter. The mesospore is brownish green or dark brown. The spore wall bears polygonal or irregularly rounded reticulations. The meshes of the reticulum are very wide as compared with those of *Z. lamellata* being 4–6 μ apart. The zygospores are typically rounded in shape, hence the specific name globosum (Fig. VII, 6). Very rarely quadrangular zygospores with thickened poles are also seen (Fig. VII, 7). Zygospores are 44–50 μ in diameter.

Aplanospores.—Very rarely one may come across spindle-shaped aplanospores 30–32 μ broad and 40–44 μ long, with reticulations on spore wall (Fig. VII, 8).

Affinities.—This species comes nearest to *Z. lamellata* from which it differs in its narrower and longer cells, fewer lamellæ in each cell, its zygospores with their broad reticulations, and the simpler structure of its aplanospores.

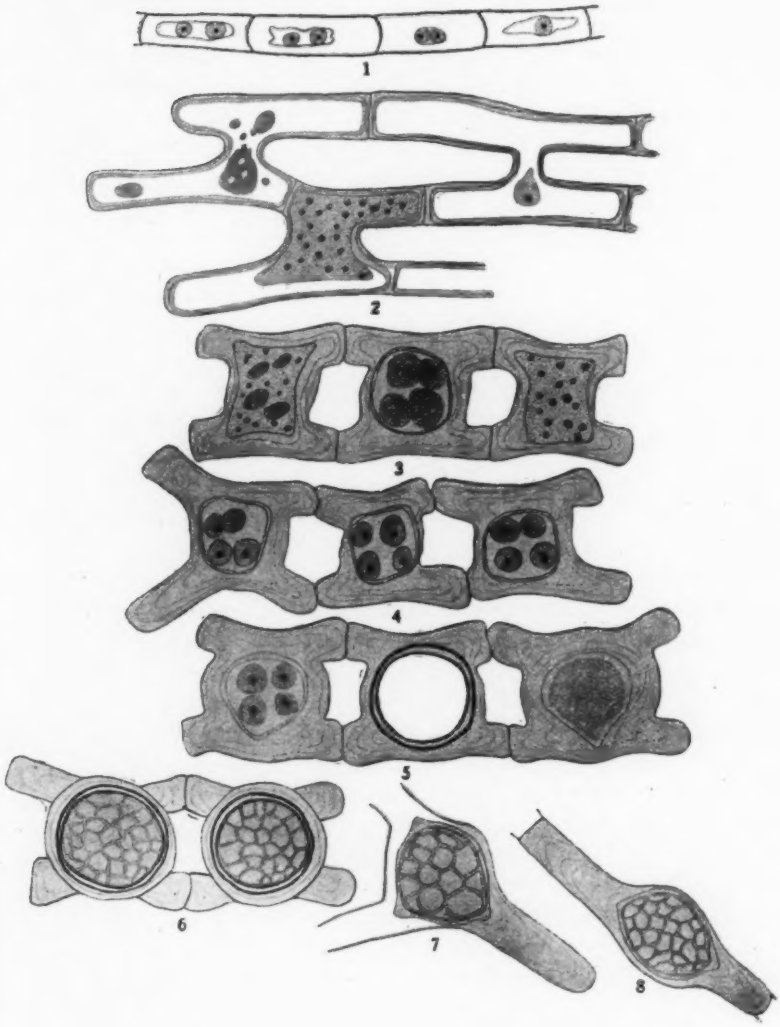

FIG. VII. *Zygnemopsis globosum* sp. nov.

1. Shows a filament, mark the single chloroplast in one cell. 2. Shows three filaments conjugating together. 3, 4 and 5. Show different stages in conjugation. 6 and 7. Show ripe zygospores with the reticulate markings. 8. Shows an aplanospore.

All figs. × 660.

Zygnemopsis globosum sp. nov. (Fig. VII).

Vegetative cells 12–14 μ broad, 36–72 μ long, each cell with one or two rounded chloroplasts. Zygospores, globose 40–50 μ broad. Spore wall with very broad reticulation on surface. Aplanospores spindle-shaped, rare, 30–32 μ broad and 40–44 μ long.

Habit.—Found mixed with a species of *Oedogonium* in a water channel near a Jhil at Tanda, district Fyzabad, U.P., on 25th March 1937.

6. *Zygnemopsis minutum* sp. nov. (Fig. VIII).

This alga was found mixed with *Zygnemopsis lamellata* Randhawa and *Zygnema Oudhensis* sp. nov. in a greyish mass free-floating in Tons Nadi near tahsil buildings, Akbarpur, Fyzabad, U.P., on 25th March 1937.

Vegetative cells are 8–10 μ broad and 36–46 μ long. Each cell contains two irregularly rounded chloroplasts. The septa are plain, and the cells have a tendency to dissociate from each other. The filaments appear like a colony of *Cylindrocystis* produced as a result of transverse separation in a horizontal plane (Fig. VIII, 1).

Reproduction.—Reproduction takes place by means of aplanospores and very rarely by means of zygospores. Conjugation is isogamous, and it appears from the absence of jointed conjugating cells that either conjugation takes place between disjointed unicells or that the dissociation of conjugating cells takes place immediately after the conjugation canals have been established. Such H-shaped pairs of conjugating cells may be seen free-floating in water. The protoplasm secretes a shining white pectic cellulose substance in a homogeneous mass as in *Zygnemopsis splendens* Randhawa (Fig. VIII, 2, 3 and 4). Zygospores are squarish when mature and retain the stumpy arm-like remains of the gametangia. Zygospores are 22–24 μ broad excluding the mucilaginous coat and inclusive of it may be as broad as 30 μ. When mature the zygospores are dark chocolate brown in colour. The spore wall bears small reticulation on surface and is composed of two layers, a thin and hyaline exospore and a thick dark brown mesospore (Fig. VIII, 5). Three-horned zygospores, produced as a result of the fusion of the terminal cell of a filament with the intercalary cell of another, are also seen (Fig. VIII, 7).

Aplanospores.—The commonest mode of reproduction in this species is by means of cudgel-shaped aplanospores. The middle part of a cell gets swollen and the sides get curved (Fig. VIII, 8). Then the spore walls appear in the swollen middle part. In early stages the aplanospores resemble those of *Zygnemopsis Iyengari* in shape (Fig. VIII, 6, 9 and 10). However in mature aplanospores, the curvature of the arms becomes very conspicuous,

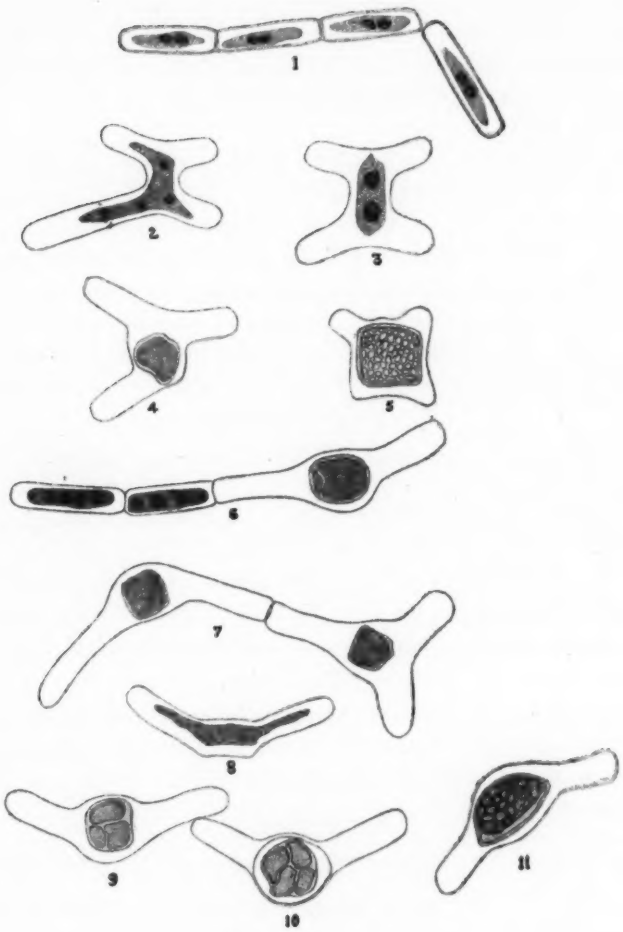

FIG. VIII. *Zygnemopsis minutum* sp. nov.

1. Shows a vegetative filament and dissociation of cells. 2 and 3. Show early stages in conjugation. 4. Shows an immature zygospore. 5. Shows a mature zygospore. Mark the reticulate sculpturing of the spore wall. 6. Shows an aplanospore. 7. Shows a three-horned zygospore along with an aplanospore. 8, 9 and 10. Show different stages in the development of an aplanospore. 11. Shows a mature aplanospore.

All figs. × 660.

and resemblance with aplanospores of some species of *Mougeotia* becomes marked. In fact the author, before he came across vegetative cells, had actually diagnosed these as aplanospores of a species of *Mougeotia*. The aplanospores are dark chocolate brown in colour and are 18–20 μ broad and 18–30 μ long. The structure of the spore wall is the same as that of zygospores (Fig. VIII, 11).

Affinities.—This delicate species of *Zygnemopsis* resembles *Zygnemopsis desmioides*. Transeau (West) nov. comb. = (*Debarya desmidioides* West) in the size and structure of vegetative cells, and the dissociation of cells and zygospores. But differences from *Z. desmidioides* are also well marked. Firstly no lamellation is noticeable in the gametangia ; secondly, the bigger size of zygospores and presence of reticulations on spore wall ; and thirdly, the presence of peculiar cudgel-shaped aplanospores. Hence, it is feasible to regard the Indian form as a new species, which is named as *Z. minutum* sp. nov.

Zygnemopsis minutum sp. nov.

Vegetative cells 8–10 μ broad, 36–46 μ long, each with two rounded chloroplasts. Reproduction by means of zygospores and aplanospores. Zygospores squarish, 22–24 μ broad, exclusive of mucilage coat. Zygospore wall composed of a thin, hyaline exospore, and a thick reticulate chocolate brown mesospore. Aplanospores cudgel-shaped, 18–20 μ broad and 18–30 μ long.

Habit.—Free-floating in Tons Nadi, Akbarpur, district Fyzabad, U.P., mixed with *Zygnemopsis lamellata* and *Zygnema Oudhensis* on 25th March 1937.

Conclusion.

Before closing, I sincerely thank Professors Ghose and Bharadwaja for so kindly lending the relevant literature, without which the proper study of these new algæ would not have been possible.

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NEW AVIAN TREMATODES OF THF SUB-SUBFAMILY *COTYLURINI* DUBOIS 1936, (FAMILY *STRIGEIDAE*, RAILLIET 1919).

By R. D. Vidyarthi, B.Sc. (Hons.), M.Sc.

(*From the Department of Zoology, University of Allahabad.*)

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1. *Apatemon indicus* n. sp.

(Fig. 1.)

The mature worms, 2·5–2·88* in length are distinctly divided into fore and hind parts. The forebody is deeply cup-shaped, 0·912 long and 0·484 broad, across the acetabulum. The hindbody of 1·872–2·972 length and 0·88–0·92 width is somewhat attenuated at both the ends and much flexed dorsally.

The oral sucker is transversely elongated and terminal, measuring 0·128–0·134 × 0·174 in size. The acetabulum is much larger than the oral sucker and situated dorsally, a little behind the equator of the forebody, measuring 0·24 in diameter. The holdfast organ is well developed and composed of an inner and outer lamellæ which usually project beyond the rim of the forebody, measuring 0·864 in length and 0·512 in width. The prepharynx is very small, visible in sections only. The pharynx is transversely oval and muscular, measuring 0·066 in length and 0·075 in width. The short œsophagus bifurcates much in front of the equator of the forebody into two simple cæca which run backwards more or less parallel to one another on either side of the acetabulum by which they are partly overlapped. Behind the latter they bend ventrally and then run ventro-laterally in the hindbody through the dense mass of the vitelline follicles terminating near the genital atrium.

The massive and kidney-shaped testes lie in tandem dorsally to the main mass of the vitellaria and the intestinal cæca. The anterior testis, 0·528–0·576 in length and 0·592–0·64 in width, is somewhat broader than long. The posterior testis, 0·624–0·672 long by 0·592–0·72 broad, is somewhat smaller. The vasa efferentia arise from the anterior concavity of their respective testes and unite just in front of the anterior testis to form the vas deferens, which dilates postero-dorsally to the second testis to form the voluminous S-shaped vesicula seminalis.

* All measurements are in mm.

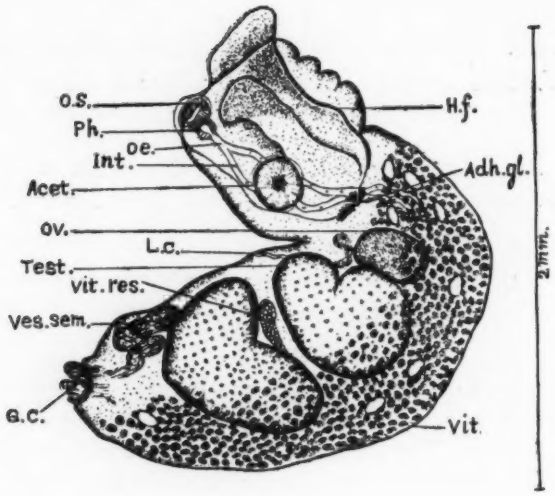

Fig. 1.

The ovary is spherical or oval, 0·272 in diameter, and situated close in front of the anterior testis and 0·128 mm. behind the body constriction. The shell gland lies between the two testes. The uterus runs forwards almost to the anterior extremity of the hindbody and there it bends on itself to continue its downward course as a slightly sinuous descending limb, which lies ventrally to the gonads. Its distal part along with the terminal portion of the ductus ejaculatorius, opens at the apex of the small, rather undifferentiated genital cone, which, however, can be protruded out of the genital atrium. The ova are large, operculate and a few in number, measuring 0·1–0·116 × 0·053 in size. The vitelline follicles are exclusively confined to the hindbody, extending from the body constriction or a little behind it to the posterior end. The great bulk of the vitelline follicles lies ventrally partly overlapping the gonads. The vitelline reservoir lies in the intertesticular space, close in front the of posterior testis.

Remarks.—Apatemon indicus n. sp. differs from the three Japanese species, *A. pellucidus*, *A. minor* and *A. fuligulæ*, described by Yamaguti in 1933, in size of acetabulum, and position and size of the gonads. It is distinguished from *A. gracilis* (Rud., 1819) Szidat 1929 and *A. graciliformis* Szidat 1929, by the size of the body, position and size of the gonads, size of the acetabulum, and size of the ova. It also differs from *A. sphærocephalus* (Brandes, 1888) Szidat 1929, in the size of the acetabulum and the pharynx, position and size of the gonads, and above all in the shape of the

forebody. It has not been compared to *A. Japonicus* Ishii 1934, the description of which is not available to me.

Host .. *Casarca ferruginea*, small intestine.
Locality .. Allahabad, U.P. (India).

2. *Apatemon casarcus* n. sp.

(Fig. 2.)

Sexually mature worms have smooth integument and, feebly developed musculature measuring 2·4–2·8 in length. The body is distinctly divided into two parts. The deeply cup-shaped forebody measures 0·88–0·96 in length and 0·672 in width, across the acetabulum ; the cylindrical hindbody is of 1·52–1·84 length and 0·704–1·28 width. The ratio in the length of the fore and hind parts is approximately 1 : 2.

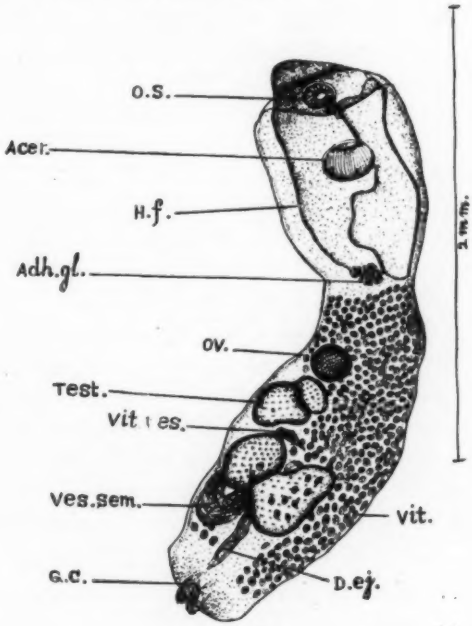

FIG. 2.

The oral sucker is oval and terminal measuring 0·1–0·165 in length and 0·135–0·15 in width. The sub-median acetabulum, 0·18–0·2 long and 0·21–0·24 broad, lies a little in front of the equator of the forebody. The holdfast organ, as in other species of this genus, consists of two well-developed lappets, which nearly occupy the whole width of the forebody,

projecting a little beyond the anterior margin of the forebody. The adhesive gland lies at the base of the holdfast organ. The prepharynx is absent; the pharynx is muscular, spherical and 0·069 in diameter. The short oesophagus forks, much in front of the acetabulum, into two simple cæca which terminate in the sub-caudal region.

The gonads occupy the middle of the hindbody. The two testes are asymmetrically bilobed and lie tandem near the dorsal side, close behind one another. The anterior testis measures 0·24–0·352 × 0·24–0·432 and the posterior 0·363 × 0·43. The vesicula seminalis is S-shaped, very voluminous and situated postero-dorsally to the hinder testis.

The transversely elongated ovary of 0·176 length and 0·256 width lies just in front of the anterior testis and approximately 0·32 behind the anterior extremity of the hindbody. The shell gland complex lies between the testes. The thin-walled uterus extends in front of the ovary upto about half the distance between it and the body constriction. The terminal portion of the uterus and the distal part of the ejaculatory duct open into a shallow genital cone, which only slightly protrudes out of the genital atrium. The vitelline follicles are exclusively confined to the hindbody, being specially well developed on the ventral side in front of the ovary. They partly overlap the gonads extending posteriorly to half the distance between the posterior margin of the second testis and the posterior extremity of the hindbody. The vitelline reservoir is inter-testicular. The dorsolateral musculature and the excretory system are feebly developed.

Remarks.—*Apatemon casarcus* n. sp. differs from all the six species so far described, in the peculiar shape of the testes. It, however, stands closest to *Apatemon fuligulæ* Yamaguti, 1933 on account of the resemblance in the form and size of its body, ratio in the length of the fore and hind parts, size of the oral sucker and pharynx and the extent of the vitellaria. But it differs from it in the large size of the acetabulum, shape and size of the testes and the ovary, and the feeble development of the dorsolateral musculature and the excretory system.

Host .. *Casarca ferruginea*, small intestine.
Locality .. Allahabad, U.P. (India).

3. *Pseudostrigea sarcogyponis* n. sp.

(Fig. 3.)

The aspinose body, 4·38–6·48 in length, is distinctly divided into fore and hind parts. The forebody is usually deeply cup-shaped with a wide opening and measures 1·408–1·712 in length and 1·264–1·52 in width.

The hind body is 2·736 × 1·0–1·312, greatly flexed dorsally, sharply constricted off from the forebody and truncated at the posterior extremity. The ratio in the length of fore and hind parts is 1 : 2. At the anterior end of the body there are two prominent suctorial pockets, one on each side of the oral sucker. The muscle fibres, originating from the thick cuticular wall of the pockets, are strongly developed forming a pair of longitudinal bundles, which are characteristically disposed in both the fore and hind bodies. They extend on either side of the acetabulum and after entering the hind body run dorso-laterally giving off at intervals a number of lateral branches postero-ventrally ; only a few fibres, however, reach the posterior extremity of the hindbody.

The oral sucker, 0·224 × 0·192, is oval and terminal in position. The prepharynx is absent. The elongated muscular pharynx, 0·12 long by

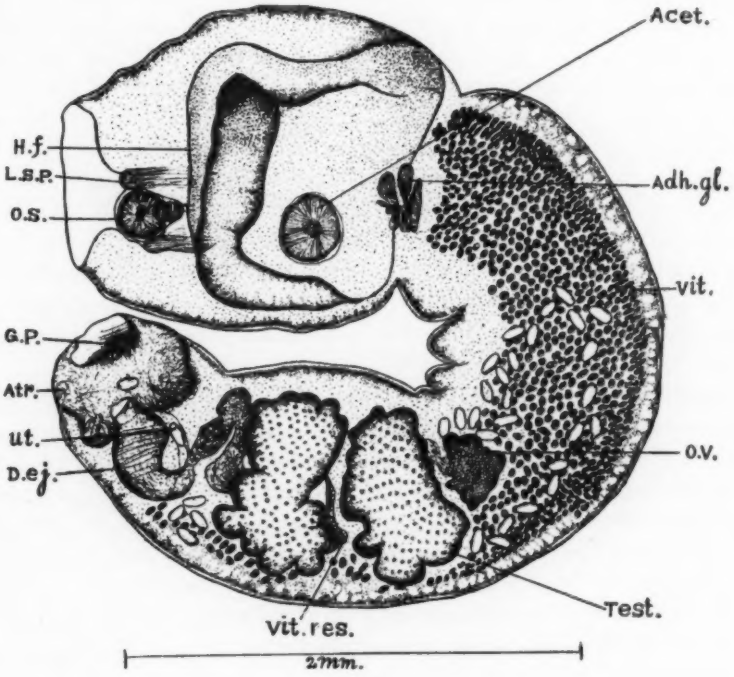

Fig. 3.

0·087 broad, is much smaller than pharynx. The simple intestinal cæca run dorsally more or less parallel to one another on either side of the acetabulum and at the junction of the fore and hind parts they pass almost ventrally,

running posteriorly through the dense mass of the vitelline follicles to near the base of the genital atrium. The more or less spherical acetabulum, 0·288–0·304 in diameter, lies immediately behind the equator of the fore body. The ratio in the diameters of the two suckers is approximately 1 : 2. The somewhat cuboidal holdfast organ, 1·088 in length and 1·024 in width, lies behind the pharynx, and consists of a broader outer and a smaller inner lobe. The basal portions of these lamellæ are thicker and broader and are fused ventrally nearly at the level of the posterior margin of the acetabulum. The adhesive gland lies median close behind the holdfast organ, at the junction of the fore and hind parts.

The genital organs, including the uterine coils and the vitellaria, are confined to the hindbody. The two large and multilobulated testes are placed one in front of the other behind the middle of the hindbody. The anterior testis is slightly smaller than the posterior one and measures 0·512–0·576 in length and 0·768–0·968 in width. The posterior testis is 0·608–0·672 × 0·768–1·072 in size. The vasa efferentia arise from the antero-ventral margin of their respective testes and join cephalad to the anterior testis to form the vas deferens which runs posteriorly, ventral to the testes. The voluminous S-shaped vesicula seminalis lies behind the posterior testis close to the dorsal body wall and continues behind into a short but well-developed ejaculatory duct, which is surrounded by a strong circular and a weak longitudinal layer of muscle fibres. The ejaculatory duct at its terminal end with the end-part of the uterus runs through the genital cone, which projects more or less distinctly into the spacious genital atrium. The latter is clearly differentiated from the surrounding parenchyma and measures 0·56 in length and 0·592 in width.

The slightly lobed ovary, 0·224–0·32 long by 0·278–0·464 broad, lies median in front of the anterior testis, dorsal to the uterine coils, large mass of the vitelline follicles, and the intestinal cæca. The oviduct arises from its postero-dorsal side and runs posteriorly after forming a few convolutions. The Laurer's canal arises from the oviduct soon after its origin. The shell gland complex is intertesticular in position. The uterus as soon as it arises turns forwards to about half way between the anterior end of the hindbody and the ovary and then bends on itself to run backwards as a more or less straight descending limb. It is filled with numerous eggs of elongated oval shape and of 0·048 × 0·112 size.

The vitelline follicles are confined to the hindbody where they lie in broad field along the ventral surface being most concentrated in the pre-ovarian region. In the postovarian region they lie in a narrow strip on the ventro-lateral side extending up to the genital cone. The large vitelline

reservoir is somewhat transversely elongated and lies immediately in front of the second testis. The excretory system shows no remarkable feature.

Remarks.—The new species differs from the type species *Pseudostrigea buteonis*, in the multilobulated condition and larger size of the testes and the ovary, greater length of its body, in the form and size of the oral sucker and the pharynx, larger size of the ventral sucker, the greater development of the dorsolateral musculature and finally in the size of the ova.

The diagnosis of the genus *Pseudostrigea* as given by Yamaguti needs modification in the light of the new form described above.

Amended diagnosis.—Family *Strigeidæ* Railliet 1919, sub-family—*Strigeinæ* Railliet 1919, sub-subfamily *Cotylurini* Dubois 1936. Body divided into deeply cup-shaped forebody and cylindrical hindbody. Hindbody approximately 2–3 times as long as forebody and curved dorsad. Vitellaria situated on ventral side from anterior end of hindbody to genital cone. Genital atrium spacious and surrounded by strong musculature. Ejaculatory duct well developed, muscular or non-muscular. Testes irregular or multilobulated one behind the other in posterior half of hindbody. Ovary globular or lobed, in front of anterior testis. Uterus extending anteriorly to about half way between ovary and body constriction. Shell-gland complex intertesticular. Laurer's canal present. Eggs elongated oval, operculate. Parasitic in carnivorous birds.

Type species—*Pseudostrigea buteonis*, Yamaguti 1933.

4. Cotylurus orientalis sp. nov.

(Fig. 4.)

The body is 3·556 in total length. It is distinctly divided into spherical to oval forebody of 1·219 length and 1·118 maximum breadth, which lies close in front of the acetabulum and cylindrical somewhat dorsally flexed hindbody, measuring 2·337 in length and 0·751 in width. The dorso-lateral musculature is fairly well developed in the hindbody. The cuticle is entirely devoid of spines. The ratio in the length of the fore and hind parts is 3 : 5.

The oral sucker is sub-terminal and spherical or oval measuring 0·134 in length and 0·167 in width. The acetabulum is situated dorsally behind the equator of the forebody. It is much larger than the oral sucker and oval, measuring 0·2 × 0·25 in size. The holdfast organ consists of four to five lobes and occupies the major portion of the post-acetabular region of the forebody. The adhesive gland is small transversely elongated and situated at the base of the holdfast organ. The prepharynx is absent. The pharynx

is transversely oval, measuring 0·083 × 0·1 in size. The œsophagus is short and the two simple cæca terminate as usual in the sub-caudal region.

The gonads occupy the middle half of the hindbody. The anterior testis, reniform in shape with the concavity pointing backwards, lies median close behind the ovary, measuring 0·236 in length and 0·467 in width. The posterior testis is much larger, somewhat star-shaped and situated 0·134 behind the anterior testis, measuring 0·568 in length and 0·467 in breadth. The vesicula seminalis is feebly developed and lies just behind the posterior testis near the dorsal surface.

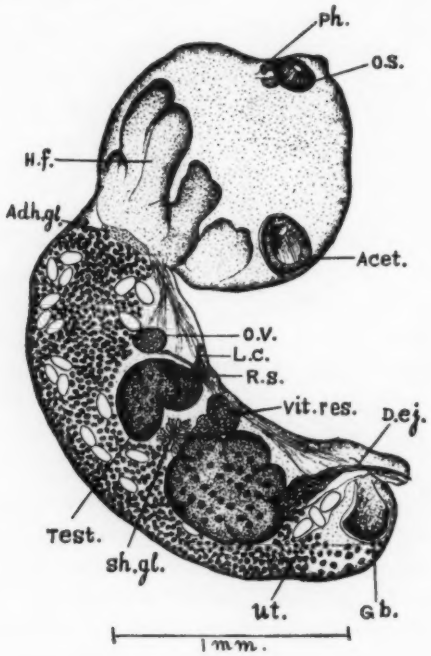

Fig. 4.

The ovary is much smaller than the anterior testis and oval to spherical in form measuring 0·134 in length, 0·167 in width. It lies somewhat dorsally closely in front of the anterior testis. The oviduct arises from its posterior margin and after a little distance gives off the Laurer's canal which opens to the exterior dorsally near the anterior margin of the anterior testis. A small receptaculum seminis is present dorsally to the anterior testis. The uterus containing 13–21 yellow, operculate ova, of 0·117 × 0·066 size,

extends forwards to near the body constriction ; its distal part along with the narrow and almost straight ejaculatory duct, opens at the apex of the highly protrusible genital cone. The large muscular genital bulb, lies ventrally to the genital cone.

The vitellaria composed of small spherical follicles are extensively developed beginning in the region of the body constriction and terminating at the posterior end of the body. The large vitelline reservoir lies close to the dorsal body wall in between the testes.

Remarks.—The new species differs from *C. platycephalus* (Creplin, 1825) Szidat 1929, *C. variegatus* (Crep., 1825) Szidat 1929, *C. erraticus* (Rudolphi, 1809) Szidat 1929, and *C. communis* La Rue 1932, in the form and smaller size of its body, position and shape of the testes, smaller size of the ovary and the presence of a receptaculum seminis. From *Cotylurus aquavis* (Guberlet, 1923) Szidat 1929, it is distinguished by the larger size of the body, shape of the forebody, form of the testes, and size of the ova. Though it bears some resemblance to *C. hebraicus* and *C. syricus* Dubois 1936, on account of the shape of the body, position and shape of the anterior testis and ovary, it differs remarkably in many features, such as the large size of the body, the shape of the posterior testis, presence of a receptaculum seminis, and the anterior limit of the vitellaria. It also differs from the other Indian species, *C. streptocorpus* Verma 1936, in the shape and size of the body, shape of the gonads, and the anterior limit of the vitellaria.

Host .. The common teal, *Nettion crecca crecca*, small intestine.
Locality .. Allahabad, U.P. (India).

I am much indebted to Dr. H. R. Mehra for his constant help and guidance. Thanks are due to Dr. D. R. Bhattacharya for providing me laboratory facilities.

EXPLANATION OF THE LETTERING.

Acet., Acetabulum ; *Adh.*, Adhesive gland ; *Atr.*, Genital atrium ; *D. ej.*, Ductus ejaculatorius ; *G.b.*, Genital bulb ; *G.p.*, Genital pore ; *H. f.*, Holdfast organ ; *Int.*, Intestine ; *L.c.*, Laurer's canal ; *L.s.p.*, Lateral suctorial pocket ; *Oes.*, Oesophagus ; *Ov.*, Ovary ; *Ph.*, Pharynx ; *R.s.*, Receptaculum seminis ; *Sh. gl.*, Shell gland ; *Test.*, Testes ; *Ut.*, Uterus ; *V. sem.*, Vesicula seminalis ; *Vit.*, Vitellaria ; *Vit. res.*, Vitelline reservoir.

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STUDY OF THE FUNGUS FLORA OF THE LAHORE SOILS.

By Hukam Chand, M.Sc.

(From the Department of Botany, Panjab University, Lahore.)

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Introduction.

During the last few decades much research has been done on the study of the microscopic fungus flora of the soil and at present the most accepted view is that all soils harbour a fairly definite flora of filamentous fungi. This fungus flora takes an active part in the soil in the microbial decomposition processes under certain conditions, apparently so energetically as to rival the importance of bacteria. Most of these studies have been carried out in America and other European countries, and the information about Indian soils as regards fungus flora is rather scanty. In India the first study of soil fungi was made by Butler (1907). He gave an account of the genus *Pythium* and some *Chytridiaceæ*. Thakur and Norris (1928) described 22 species from Madras soils with special reference to their power of cellulose decomposition and ammonification. Mason (1928) has described 4 species of *Aspergillus* and one species of *Acrothecium* isolated from paddy soil by Rhind in Burma. Chaudhuri and Sachar (1934) have given full description of the 32 species isolated from soil samples taken at Lahore. They have described and named four new species and one new variety. Recently Galloway (1935) has given generic descriptions of soil fungi taken from Pusa and certain hilly districts in North India. Mahju (1933) and Ginai (1936) have described the fungus flora occurring on the dungs of various animals in the Punjab, many of which are common soil forms.

Experimental.

For this work a manured and an unmanured plot were selected in the Government College Botanic Garden, Lahore. Three to four inches of soil from the surface was removed before sampling was done, the technique used being the one suggested by Brierley (1923). Though several media were tried, Conn's starvation medium as suggested by Brierley (1923) proved

most useful for quantitative and qualitative studies and has been used throughout. It consists of dipotassium phosphate—1 g.; sodium asperginate—1 g.; glycerine—10 c.c.; water—1 litre to which 30 g. of agar is added. The pH of the medium is adjusted between 4 and 5. Eight plates in parallel were prepared for the manured and unmanured plots several times during the course of the work. The plates were incubated at 25°C. After the colonies appeared, isolations of the different forms were made by Brown's hyphal-tip method.

Morphological Examination.

The detailed morphological examination of each colony growing on a petri-dish used to begin on the third day and continued for a week or so. After making a preliminary survey for the general features like colony colour, floccosity etc., microscopic mounts were prepared to determine the detailed structure and measurement of essential cells.

Staining.

Preparations were stained either in erythrosine following the methods described by Rawlins (1933) or in cotton blue. As sometimes erythrosin preparations faded the material to be stained was first put in a mordant of 2·5 per cent. $Al_2(SO_4)_3$ solution before staining. This gave very satisfactory results. Preparations were mounted in pure glycerine and ringed with Canada Balsam.

List of Species Isolated.

These are *Rhizopus nodosus*, *Mucor botryoides*, *Choanephora* sp., *Cunninghamella echinulata*, *Sordaria macrospora*, *Chætomium globosum*, *Fusicoccum* sp., *Cytospora* sp., *Monilia* sp., *Trichoderma lignorum*, *Trichoderma glaucum*, *Spicaria* sp., *Stirochæte* sp., *Spondylocladium fumosum*, *Heterosporium allii*, *Helminthosporium anomalum*, *Alternaria malvæ*, *Alternaria* sp., and *Stemmaria terrestris*.

In the following table a complete list of the Indian soil fungi so far isolated is given along with the name of the investigator.

Name of Fungi	Recorded by
Rhizopus nigricans	Author, Chaudhuri & Sachar, Galloway and Thakur & Norris
R. nodosus	Author
R. arrhizus	Chaudhuri & Sachar and Galloway
Mucor circinelloides	Chaudhuri & Sachar
M. racemosus	Thakur & Norris
M. glomerula	Thakur & Norris
Choanephora sp.	Author
C. echinulata	Author and Galloway
Cunninghamella verticillata ..	Chaudhuri & Sachar
Syncephalastrum sp.	Galloway
Chætomium Indicum	Galloway
Sordaria sp.	Galloway
S. macrospora	Author
Byssochlamys sp.	Galloway
Phoma sp.	Galloway
Fusicoccum sp.	Author
Cytospora sp.	Author
Melanconium sp.	Galloway
Monilia sp.	Author and Galloway
M. koningi	Chaudhuri & Sachar
Cephalosporium sp.	Chaudhuri & Sachar
C. acremonium	Thakur & Norris
Trichoderma album	Thakur & Norris
T. koningi	Chaudhuri & Sachar

Name of Fungi	Recorded by
T. lignorum	Author and Galloway
T. glaucum	Author
Oidium lactis	Thakur & Norris
Aspergillus fumigatus	Chaudhuri & Sachar, Galloway and Thakur & Norris
A. nidulans	Chaudhuri & Sachar, Galloway and Thakur & Norris
*A. niger**	Chaudhuri & Sachar, Galloway and Thakur & Norris
A. calyptratus	Chaudhuri & Sachar
*A. terreus**	Chaudhuri & Sachar and Galloway
A. Sachari	Chaudhuri & Sachar
A. flavipes	Chaudhuri & Sachar and Galloway
A. sydowi	Chaudhuri & Sachar and Galloway
A. versicolor	Chaudhuri & Sachar
A. candidus	Chaudhuri & Sachar and Thakur & Norris
A. humicola	Chaudhuri & Sachar and Thakur & Norris
A. fuscus	Galloway and Thakur & Norris
A. repens	Thakur & Norris
*A. flavus**	Thakur & Norris
A. ustus	Galloway
A. ochraceous	Galloway
*A. tamarii**	Galloway
A. chevalieri	Galloway
Penicillium terrestre	Chaudhuri & Sachar

Name of Fungi	Recorded by
P. pinophillum	Chaudhuri & Sachar
P. oxalicum	Thakur & Norris
P. viridi-varians	Chaudhuri & Sachar
P. glaber	Thakur & Norris
P. digitatum	Thakur & Norris
P. glaucum	Thakur & Norris
Spicaria silvatica	Chaudhuri & Sachar
Spicaria sp.	Author
Sporotrichum roseum	Thakur & Norris
Botrytis cinerea	Thakur & Norris
Citromyces glaber	Thakur & Norris
Verticillium glaucum	Thakur & Norris
Acrostalagmus sp.	Thakur & Norris
Cephalothecium roseum	Author and Chaudhuri & Sachar
Dematium	Galloway
Botryotrichum sp.	Galloway
Mesobotrys sp.	Galloway
Cladosporium herbarum	Chaudhuri & Sachar and Galloway
Epochnium sp.	Galloway
Helminthosporium sp.	Chaudhuri & Sachar
H. anomalum	Author
H. sativum	Galloway
H. tetramera	Galloway
*Acrothecium lunatum**	Chaudhuri & Sachar and Galloway

Name of Fungi	Recorded by
Spondylocladium sp.	Author
Heterosporium	Author
Alternaria tenuis	Galloway
A. humicola	Chaudhuri & Sachar
Alternaria sp.	Author
Torula sp.	Galloway
Trichosporium sp.	Galloway
Coniosporium sp.	Galloway
Stirochæte sp.	Author
Fusarium sp.	Chaudhuri & Sachar, Galloway and Thakur & Norris
F. oxysporium	Chaudhuri & Sachar
Myrothecium sp.	Galloway
Dictyochæte sp.	Chaudhuri & Sachar
Stysanus stemmonites	Galloway
Stemmaria terrestris	Author and Chaudhuri & Sachar
Hypha sp.	Chaudhuri & Sachar

* These have been described by Mason (1928) from isolations from the Burma paddy soils, made by Rind. Butler (1907) has also described the soil *Pythiaceæ* as already mentioned (*Pythium de-Baryanum, P. intermedium, P. vexans, P. proliferum, P. rostratum* and *P. monospermum*).

Besides the above forms isolated from the soil, there are many forms which are found both in the soil and on dungs of various animals. For a list of these dung fungi recorded in India reference to papers by Mahju (1933) and Ginai (1936) should be made. Amongst the undoubted soil organisms, isolated by the above workers from dung, may be mentioned the following :— (1) *Mucor mucedo*, (2) *M. griseosporus*, (3) *Syncephalis sphæriea*, (4) *Aspergillus flavus*, (5) *Sordaria macrospora*, (6) *Sordaria coprophila*, (7) *Sordaria*

winteri, (8) *Sordaria decipiens*, (9) *Chætomium spirale*, (10) *C. globosum*, (11) *Sporomia minima*, (12) *Stysanus stemmonitis*, (13) *Coremium* sp., (14) *Isaria brachiata*, (15) *Torula convoluta*, (16) *Oedocephalum glomerulosum*.

Intensities of Various Genera.

Waksman (1927) and other workers have pointed out that *Aspergillus* is more abundantly found than *Penicillium* in the warmer soils, the latter being predominantly found in cooler climates. Chaudhuri and Sachar (1934) and Galloway (1936) have also found the same to be true. The present author has also found that to be true only to a certain extent, specially regarding the number of species of each form. Working on the intensity of these two forms both in the manured and the unmanured plot, the author found that it varied according to the season in both the plots. From early spring to the beginning of moist hot weather in July–August, there was always found a great deal of difference in the number of *Aspergilli* and *Penicillii* present in the soils. With the drop in temperature, the number of *Penicillii* rose high and by the end of September, the *Aspergilli* and *Penicillii* counts both of the unmanured and manured soils equalled and at the approach of winter, the number of *Aspergilli* fell down very quickly but not so that of the *Penicillii*. The samplings in November clearly showed that *Penicillii* number increased in winter.

Conclusion.

The present investigations show that the soil fungi are almost cosmopolitan. If detailed work is pursued, no doubt practically all the genera and many of the species reported from Europe and America could be found. Regarding the total number of the organisms, it has been found that the temperature plays a very important part as also the moisture contents of the soil. *Aspergilli* and *Penicillii* occur in the largest number in the soil and either the former or the latter will predominate depending upon whether the temperature is high or low.

About twenty species of fungi have been recorded by the author. Of these some are new records.

Before concluding, the author takes the opportunity of thanking Dr. H. Chaudhuri and Dr. J. Singh for their kind interest in this work and many helpful suggestions and general guidance.

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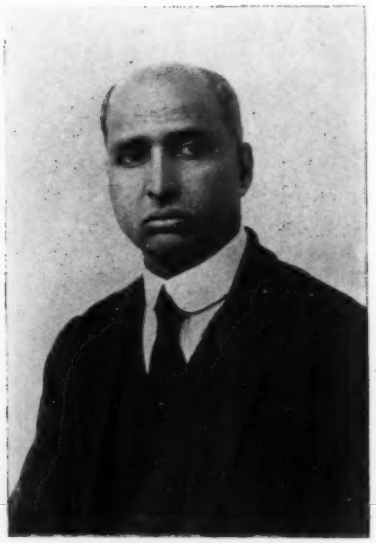


RAI BAHADUR DR. SHIV RAM KASHYAP

OBITUARY NOTICE.

Rai Bahadur Dr. Shiv Ram Kashyap.

(1882-1934.)

RAI BAHADUR DR. S. R. KASHYAP, B.A. (Cantab.), D.Sc. (*Honoris Causa*, Panjab), I.E.S., F.A.S.B., Professor of Botany, Government College, Lahore, and of the Panjab University and Honorary Professor of Botany, Hindu University, Benares, died suddenly at Lahore, on the 26th November, 1934, of heart-failure. Even half an hour before his death, he was attending to his work with his characteristic thoroughness.

To-day his country is the poorer by the loss of one of its best-known and best-beloved intellectuals. As a scientist, he was respected all the world over and as a teacher he will be mourned by a host of admiring students all over the country, many of whom are holding University Chairs in Botany and other important appointments. For ever, he will be looked upon as one of the chief makers of modern Indian Botany. His pioneer researches on Himalayan Liverworts will go down to posterity as a great scientific achievement which will keep alive his memory for ever.

In his early life, Kashyap had a brilliant and remarkable academic career which can seldom be rivalled. Born on 6th November 1882 at Jhelum of a family with a long record of meritorious military services, he matriculated from the Panjab University in 1899. In 1900, he joined the Medical School at Agra and received his Medical Diploma in 1904, topping the list of successful candidates and winning the First Medal. He then served for two years in the Medical Service of the United Provinces.

While still a student of the Medical School he appeared as a private candidate for the Intermediate science examination of the Panjab University and not only succeeded in passing the examination in the first division but stood at the top of the list in the University. He was offered a university scholarship but this he declined to accept and went on with his studies at the Medical School. In 1906, while serving in the Medical Department of the United Provinces, he again appeared as a private candidate for the B.Sc. examination of the Panjab University and again topped the list of successful candidates. In the same year he resigned his post in the Medical Service and was appointed Assistant Professor of Biology at Government College, Lahore. In 1909, he passed the M.Sc. examination in Botany, again getting a very high first class and standing first among the M.A. and

M.Sc. candidates in the University. As a result he was awarded the much-prized Arnold and Maclagan Gold Medals of the University.

In 1910, he went to Europe and joined the Cambridge University from where, in 1912 he took his Honours Degree in the Natural Science Tripos.

On his return home, Professor Kashyap was appointed Professor of Botany at the Government College, Lahore, in the senior grade of the Provincial Educational Service and was promoted to the Indian Educational Service in 1920. In 1919, when the Honours School in Botany was organised by the Panjab University, he was appointed University Professor of Botany, in which capacity he continued to serve the cause of education until his death.

He had been an elected Fellow of the University for many years and the Dean of the Science Faculty for a long time. He was also a member of the Syndicate and in 1931 officiated as Dean of University Instruction for some months. He had been for several years a member of the Science Faculty and of the Boards of Studies of several other Universities also, such as Agra, Lucknow and Benares. Professor Kashyap was the first systematic botanist to preside over the annual deliberations of the Indian Science Congress in 1932, ever since it was founded in the year 1914. It was the highest honour that the Indian scientists could confer on him. In recognition of his valuable contributions, the Panjab University, in 1933, conferred on him, *Honoris Causa*, the Degree of Doctor of Science.

He was the first Secretary of the Indian Botanical Society, which was founded in 1920, and did all the spade work in its organisation. He was its President in 1925 and was the Editor-in-Chief of its organ, the *Journal of the Indian Botanical Society*. He was also an Advisory Editor of *Chronica Botanica*, published from Holland. He was elected President of the Botany Section of the Indian Science Congress in 1919 when the Congress met at Bombay. In 1934, he was elected a Fellow of the Indian Academy of Sciences.

He contributed numerous papers on various groups of the Vegetable Kingdom. His contributions to three subjects—the sexual generation of Equisetum, the Liverworts of the Western Himalayas and the Flora of Tibet—however, stand out above all others. One of his very first papers appeared in 1919, in which he described the structure and development of the sexual generation of *Equisetum debile*, one of the Indian members of the family of horsetails, growing at Lahore, which was quite different from what was known up to that time in many species worked out by European and American investigators. The account was so different that some people

even made adverse remarks in the beginning but later they all agreed when they saw the specimens. In 1917, Prof. Kashyap suggested that the sexual generation of other species would also show a similar structure as seen in the Indian species if certain conditions with regard to space and nourishment were fulfilled. Later work has shown how prophetic his prediction was.

The second subject of Prof. Kashyap's investigations has been the little group of Liverworts or *Hepaticæ*. His contribution to the knowledge of the West-Himalayan Liverworts has been very great and this may be regarded as his chief subject. He described 4 new genera and over 30 new species of Liverworts. When his first paper on them was published Professor Goebel, of Munich University, one of the leading botanists of the World, wrote to him : " You have struck a gold vein in the Western Himalayas and made a most valuable addition to our knowledge of Liverworts." Professor (now Sir John) Farmer wrote, " I cannot refrain from congratulating you on the advance towards our knowledge of these interesting plants which you have been able to make."

His contribution to the Theory of Evolution by Reduction, in this group is very important and has been highly appreciated in Europe and America. He not only greatly expanded this theory and placed it on a strong basis but actually followed out the various lines of evolution. Dr. Cavers wrote to him, " I shall have to re-write the portion of ' Interrelationships ' dealing with the Marchantiales." Recently he published a monograph on the Liverworts of the Western Himalayas and the Punjab Plain. This is a very valuable piece of work and fills a great gap in the botanical literature of India.

The third subject to which Prof. Kashyap very largely contributed is the Flora of the Western Himalayas and Western and Central Tibet. He was a great traveller and probably knew more than anybody else about this interesting country. He crossed the Himalayas into Tibet at nine different places throughout its length and several times at some places. Among the regions visited by him are Ladak, the sources of practically all the rivers of the Panjab, the world-famous sacred country of Mountain Kailas and Lake Manasarovar, the sources of the Ganges and the Jumna, the country in the neighbourhood of Kanchenjunga, Gyantse, etc., etc. It may be said of him that he knew the Himalayas as one knows his own home. He brought back a very large collection of plants from these places, many of which have not been recorded from that country so far. Besides adding to the botany of these regions, he even extended their geographical

knowledge. The exploration of the Himalayas and the study of its vegetation were his life's passion. It may not be known to all that years back when far out on the other side of the Himalayas, he fell ill and had to be brought down almost in an unconscious state. But that could not keep him back from further explorations in the following years. Last year, when up on the Himalayas, he again became seriously ill and had a bad attack of heart trouble. This year, he slightly recovered from its effects and was planning yet another trip to the Himalayas. With him, his work came first and if any one remonstrated with him for working so hard in his failing health, he would say " Why, my life is not more important than my work." Such was his love for his work. Those of us who had the rare good fortune of working with him as colleagues for a number of years look upon his death as a personal loss. A distinguished scientist, a pioneer botanist, a great teacher, and an indefatigable worker, an untiring Himalayan explorer, yes, he was all that but much more—he was, on top of everything else, a sincere friend and a generous helper to all his colleagues and pupils. Richly endowed with qualities of head, he achieved his tremendous popularity even more by his unique qualities of heart. He possessed in an extraordinary degree that sweet reasonableness which stamped him out at once as a gentleman and a man of true culture. All those who like me came in close personal contact with him, at once felt the subtle charm and magnetism and human warmth of his presence.

Such was the man who is deeply mourned to-day by a very wide circle of grief-stricken friends and admirers.

The following is a list of the publications of Professor Kashyap :—

(1) Notes on species of *Euglena* (*Records of the Indian Museum*, Calcutta, April 1908). (2) Structure and development of the Prothallus of *Equisetum debille* (*Annals of Botany*, London, January 1914). (3) Notes on New and Little-known West Himalayan Liverworts, No. 1 (*New Phytologist*, Cambridge, 1914). (4) Notes on New and Little-known West Himalayan Liverworts, No. 2 (*New Phytologist*, Cambridge, 1914). (5) Notes on New and Little-known West Himalayan Liverworts, No. 3 (*New Phytologist*, Cambridge, 1915). (6) The genus *Riccia* and the origin of the Pteridophytes (*Lahore Phil. Soc.*, June 1915) (*Proc.* for 1915-16). (7) Notes on *Targionia hypophylla* (*New Phytologist*, Cambridge, 1917). (8) Notes on *Equisetum debille* (*Annals of Botany*, London, 1917). (9) Liverworts of the Western Himalayas and the Punjab Plain, No. 1 (*Jour. Bomb. Natur. Hist. Soc.*, 1916). (10) Liverworts of the Western Himalayas and the Punjab, No. 2 (*Jour. Bomb. Natur. Hist. Soc.*, 1917). (11) Notes on the inflorescence of *Zea Mays* (*Lahore Phil. Soc.*, June 1918) (*Proc.* for 1917-20). (12) Abnormal number of needles in the shoots of *Pinus longifolia* (*Jour. Ind. Bot.*, 1919). (13) Presidential Address to the Botany Section of the Indian Science Congress at Bombay in Jan. 1919 (*Proc. As. Soc. Beng.*, New Series, Vol. 15, 1919, No. 4, The relationships of Liverworts, especially in the light of some recently discovered Himalayan Forms). (14) The Andrœcium of *Plagiochasma appendiculatum* L. and *P. articulatum* Kash. (*New Phytologist*, 1919). (15) Variability in some Himalayan Liverworts

(*Lahore Phil. Soc.*, February 1917) (*Proc.* for 1917-20). (16) Distribution of Liverworts in the Western Himalayas (*Lahore Phil. Soc.*, November 1919) (*Proc.* for 1917-20). (17) Floating Islands of Riwalsar (*Jour. Ind. Bot.*, April 1920). (18) Notes on the distribution of Liverworts in the Western Himalayas, Ladak and Kashmir (*Jour. Ind. Bot.*, May 1921). (19) Some observations on *Cycas revoluta* and *C. circinalis* growing in Lahore (*Jour. Ind. Bot.*, June 1921). (20) Notes on some Foreign Plants which have recently established themselves about Lahore (*Jour. Ind. Bot.*, December 1932). (21) A contribution to the Life-history of *Aneura indica* St. (*Jour. Ind. Bot.*, December 1922, jointly with S. K. Pande). (22) A long-lost Liverwort (*Monoselenium tenerum*) (*Jour. Ind. Bot. Soc.*, 1923). (23) Two Indian species of the genus *Notothylas* (*Lahore Phil. Soc.*, November 1923, Vol. 4). (24) Some abnormalities in the flowers of *Cannabis sativa* (*Jour. Ind. Bot. Soc.*, 1925, Vol. 4, No. 6). (25) Abnormal sporophylls in the male cone of *Cycas circinalis* (*Jour. Ind. Bot. Soc.*, Vol. 4, Nos. 9 and 10). (26) The Vegetation of Western Himalayas and Western Tibet in relation to their climate (*Jour. Ind. Bot. Soc.*, Vol. 4, Nos. 9 and 10, 1925). (27) The colour in the flowers of *Potentilla argyrophylla* (*Abs. Ind. Sci. Cong.*, 1926). (28) *Salsola fœtida* with special reference to its galls (*Proc. Lahore Phil. Soc.*, Vol. 5, 1925-26). (29) Replacement of fertile shoots by vegetative shoots in *Euphorbia tibetica* Boiss. (30) A new species of *Petalophyllum P. indicum*, Kashyap, from Lahore (*Jour. Ind. Bot. Soc.*, 1928. Vol. 7, No. 1). (31) A study of *Dumortiera* (jointly with Mr. Brij Lal Sethi) (*Abs. Ind. Sci. Cong.*, Madras, 1929; Botany Section *As. Soc. Beng.*). (32) Observations on the flora of the Upper Chandra Valley and Spiti (*Abs. Ind. Sci. Cong.*, Madras, 1929, *As. Soc. Beng.*). (33) Some geographical observations in Western Tibet (*Jour. and Proc. As. Soc. Beng.*, 1929, Vol. 25, No. 1). (34) Liverworts of the Western Himalayas and the Punjab Plain, Part I (Punjab University Publication, 1929). (35) Liverworts of the Western Himalayas and the Punjab Plain, Part I, Supplement (Punjab University Publication, 1933). (36) Liverworts of the Western Himalayas and the Punjab Plain, Part II (Punjab University Publication, 1933). (37) The Liverwort flora of Sikkim, Read at the Ind. Sci. Cong., Allahabad, January 1930 (*As. Soc. Beng.*, Bot. Section). (38) Notes on the flora of Central Tibet, Read at the Ind. Sci. Cong., Allahabad, January 1930 (*Abs. Bot. Sec., As. Soc. Beng.*). (39) Some abnormal cones in *Equisetum debille* (*Jour. Ind. Bot. Soc.*, Vol. 9, No. 4). (40) Some peculiar cones and microsporophylls of *Cycas circinalis* (*Jour. Ind. Bot. Soc.*, Vol. IX, No. 4). (41) *Stellera chamœjasme* Linn. (*Jour. Ind. Bot. Soc.*, 1930, Vol. IX, No. 4). (42) Acrogynous Liverworts of the Western Himalayas (*Malaviya Commemoration Vol.*, 1932). (43) Some aspects of the Alpine vegetation of the Himalaya and Tibet with appendix—List of plants in the Herbarium of the Botany Department, Government College, Lahore, collected by Prof. S. R. Kashyap beyond the main Himalayan range, Pres. Address to the Nineteenth Indian Science Congress at Bangalore (*Proc. of the 19th Ind. Sci. Cong.*, 1932). (44) Autonomous movement in the leaves of *Curculigo recurvata*, Dryand. (*Current Science*, Vol. I, No. 1). (45) Some more peculiarities in the male cone of *Cycas circinalis* collected at Lahore in 1932 (*Ind. Sci. Cong.*, Patna, 1933, Abs.). (46) An account of a Journey to the Gangotri Glacier (*Urusvati Journal*, 1933). (47) Jointly with P. N. Mehra, Dichotomous branching in the leaves of *Pleopeltis simplex* Sw. (*Current Science*, 1934, Vol. III, No. 2). (48) Flora of the Central Punjab (To be published shortly by the Punjab University).

H. CHAUDHURI.

PROFESSOR K. K. MATHUR

OBITUARY NOTICE.

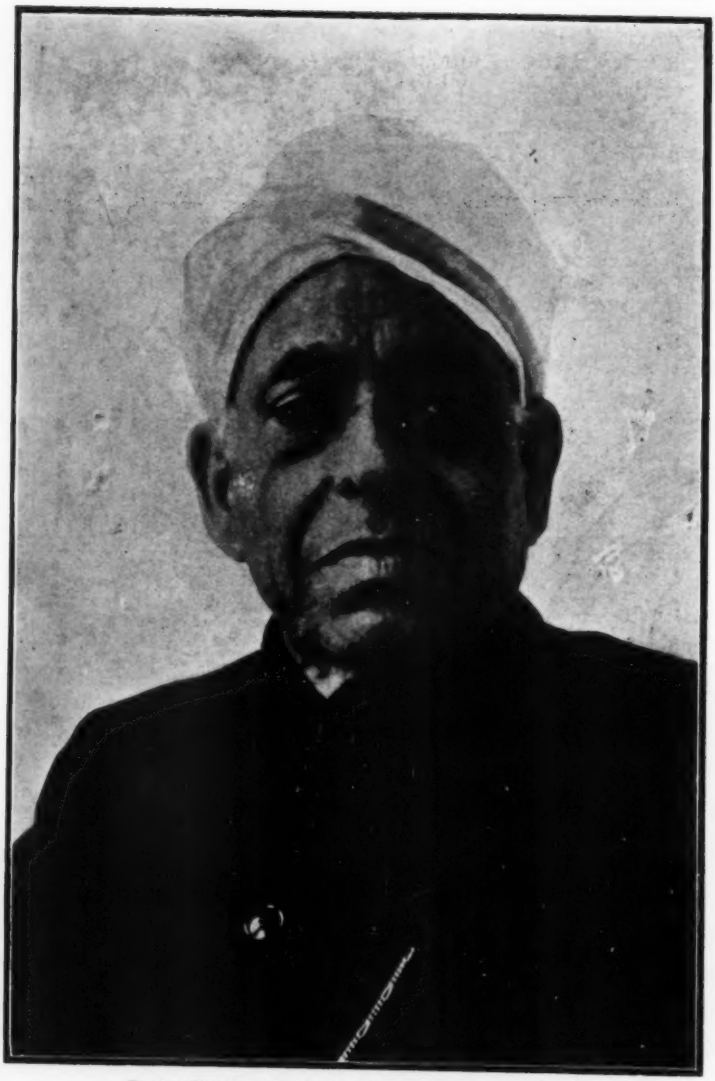
Professor K. K. Mathur.

We deeply regret to record the premature death on 18th July 1936, at the early age of 43, of Prof. K. K. Mathur, Professor of Geology, Benares Hindu University, and one of the Foundation Fellows of the Indian Academy of Sciences. After a distinguished educational career in India and abroad, Mr. Mathur was appointed Professor of Geology in the Benares Hindu University, which post he held with great distinction for these 15 years. Only about a year before his death, Prof. Mathur was elevated to be the Principal of the Science College in the Hindu University; and it is sad to think that Death snatched him away so soon and deprived the institution of the full benefits of his ripe experience and mature judgment.

In the field of Indian geology, the late Prof. Mathur will long be remembered for his valuable work on the petrology of the Deccan traps, his paper on the "Magmatic Differentiation in Mount Girnar" (*Jour. Geo.*, 1926, **34,** No. 4) being an outstanding contribution. In the course of his microscopic study of the rocks from this area, Prof. Mathur was able to recognise a new member of the Hastingsite group of amphiboles (which he called *girnarite*), the physical, chemical, and optical properties of which he described in a paper published in 1931. From the Presidential chair of the Geology Section of the Indian Science Congress, which he was invited to occupy in January 1934, Prof. Mathur delivered a very lucid and suggestive address on "Some problems of petrogenesis in the Deccan trap"; and there is no doubt that, if he had been spared for some more years, he would himself have contributed largely to the elucidation of many of these problems.

By his genial and courteous temperament, the late Prof. Mathur had gathered round him a band of young and enthusiastic workers and was thus responsible for building up the reputation which the Benares Hindu University now enjoys as a centre of geological instruction and research. By his simplicity of habits and sincerity of purpose, he had endeared himself to a large circle of friends and relations to many of whom his death comes with all the poignancy of a personal bereavement.

L. R.



DEWAN BAHADUR DR. L. K. ANANTHAKRISHNA IYER

OBITUARY NOTICE.

Dewan Bahadur Dr. L. K. Ananthakrishna Iyer.
(1861–1937.)

THE news of the death of Dewan Bahadur Dr. L. K. Ananthakrishna Iyer, which sad event occurred on February 25, 1937, was received by his numerous friends and admirers with great sorrow. To the last moment, he was apparently in the enjoyment of excellent health and spirit and those that met him at the Indian Science Congress Session in Hyderabad, could hardly have imagined that his end was so near.

Dr. Ananthakrishna Iyer was a great pioneer in the younger branches of biological sciences, namely, Anthropology and Ethnology, and his indefatigable industry and remarkable activity in field work and in his study have been the admiration of all who knew him intimately. The results of his labours, besides diffusing general knowledge of the customs and manners of the different groups and communities of South Indian people have created a general interest in the science itself, which has made a rapid progress under his inspiring example and guidance. His life comprehended well-nigh forty years of ethnographical research. The brief bibliography of his more important works given at the end of the sketch represents a record of honourable and distinguished achievement. We stand too near the period during which Dr. Iyer laboured to be able to assess the real value of his accomplished work. But we have no doubt that as Anthropological studies progress in India, his contributions will be regarded as the great foundation on which the superstructure has to be built.

Dr. Ananthakrishna Iyer born of an orthodox Hindu family in Palghat, received his early education in the local schools. From his early boyhood, he had shown considerable zeal and intelligence in his studies which were noticed by his school teachers. After a distinguished career in the local English schools from which he matriculated, he proceeded to Calicut for completing his undergraduate course. At Kerala Vidyasala, he came under the notice of Mr. Barrow, the Principal of the College, with whom he was later destined to work as a colleague in the Palghat College. Dr. Ananthakrishna Iyer obtained his B.A. degree of the Madras University, in science, and during his studies in the Madras Christian College, he cultivated the friendship of Dr. William Miller, a missionary Principal, who was known alike for his piety and public zeal, and whose contributions to the spread of higher education in India could hardly be surpassed.

Dr. Ananthakrisnha Iyer's official career ranged over a very wide field and although he shifted from one appointment to another he had the power and capacity to enrich his experience. He had, therefore, opportunities of coming into intimate contact with numerous people who were his colleagues or superior officers and from his early days he developed habits of close and critical observation of men and manners. This experience formed the foundation of his later interest in anthropological researches. Dr. Ananthakrishna Iyer was a man slow in forming his opinions and had a very large measure of scientific scepticism which accounts for the great rigour with which he applied the scientific methods to his studies and for the great precision and clearness of his descriptions.

After a distinguished career as science teacher at the Ernakulam College, Dr. Ananthakrishna Iyer in whom the Government of Cochin had recognised some of the lineaments of the future anthropologist appointed him to investigate the cultural and racial problems of the different communities inhabiting that small but extremely interesting State. Dr. Ananthakrishna Iyer immediately found himself in his element and addressed himself to his task with his characteristic energy and enthusiasm. Though he was not provided with all the equipments necessary for his investigation, still this deficiency was amply supplied by his insight and his perseverance, and the results that he has accomplished under such circumstances have received the great approbation from his European colleagues and friends. In 1908, his first contribution on the "Cochin Tribes and Castes" was issued by the Government and later was followed by a second volume. The publication of these two sumptuous volumes elicited the warmest encomiums from Dr. John Beddoe and M. Bougle who were impressed at once by the painstaking industry of the author and the care with which a great mass of facts had been sifted and recorded in true scientific spirit.

On the conclusion of his anthropological studies, Dr. Ananthakrishna Iyer was deputed to organise a Zoological Garden and Museum for the Cochin State and although he was entirely new to this branch of knowledge, still his capacity for acquisition of fresh knowledge and his power of utilising it were responsible for the creation of an exceedingly interesting Institute in Trichur, whose popularity and educational value in the collection and arrangement of the exhibits attracted the notice of the Government as well as of the foreign visitors to the State. By 1913, the reputation of Dr. Ananthakrishna Iyer as a first-rate anthropologist was established and his place among the leaders of science was secured. In 1916, the University of Madras appointed Dr. Ananthakrishna Iyer as Reader to deliver a series of lectures in Ethnology.

During the eight years from 1912–20, Dr. Iyer was engaged in further studies on the peoples of Cochin. The original plan was to complete the Cochin survey in three volumes, the last one being devoted to an Anthropometric enquiry. This was interrupted by an independent investigation on the Syrian Christians of Malabar, Cochin and Travancore. The monograph bearing the same name was published by the Cochin Government Press in 1924 after Dr. Iyer's retirement from the State.

In 1921, Dr. Ananthakrishna Iyer was invited by the University of Calcutta to accept the post of Lecturer in Ethnology to conduct Postgraduate work in the subject and also to organise the Anthropological and Ethnological Department. He remained the Head of the Department and the Chairman of the Board of Studies in Anthropology till his retirement in 1932. Even here his activities were never confined to one place or locality: after the lectures of the usual sessions he took the students to backward tracts to train them in field-work and practical investigations.

In 1924, the Government of Mysore appointed him Officer-in-charge of Ethnographic Survey, which he used to carry on during his long vacation. His work in Mysore comprehended a critical survey of 104 tribes and castes published in four superb volumes, most beautifully edited and illustrated. These four volumes to which prefaces and introductions were contributed by European savants such as Baron von Eickstedt, Dr. Marett and others, will always remain a monumental contribution to the descriptive science of South Indian Anthropology, and should provide the basis for future anthropologists for further investigations. In the first volume which was curiously published last, he adopted a more extended canvas and discussed in great detail the ethnology of Mysore in the South Indian setting. It might be regarded as one of the finest and most exhaustive treatises on South Indian Ethnology.

Early in 1934, Dr. Ananthakrishna Iyer received invitations to lecture at a number of Universities in Europe. He spent about five months out of India, during which he visited Naples, Rome and Florence in Italy, and delivered addresses on South Indian ethnographic and anthropologic topics which were universally admired and appreciated. Some of the topics that he dealt with like " Black Magic in India ", " Primitive Culture in Southern India " were characterised by remarkable scholarship and wide researches, and in appreciation of his distinguished service to the science of Anthropology, the University of Firenze awarded to him the University Medal. In Paris, he addressed the Members and Fellows of the Anthropological Institute and School of Indology under the Presidency of Dr. Sylvain Levy, and immediately after this he was invited to Oxford to give at the Pitt-Rivers

Museum a lecture on South Indian Culture. He toured widely both in Austria and in Germany and in every place he visited he was welcomed by the Learned Societies with great enthusiasm and was earnestly solicited to address the various Institutions on the different cultural and racial aspects of South Indian Anthropology. Of course, his reputation as the distinguished author of some of the great works of Indian Anthropology had already preceded him and his numerous friends who had known him through his works were greatly rejoiced at meeting him. Dr. Ananthakrishna Iyer was elected Vice-President of the Section of General Ethnology and Sociology at the International Congress of Anthropological and Ethnological Sciences held in London in 1934. His long record of contributions to Anthropology and Ethnology was recognised by the Comite d' Organization, and he was elected unanimously to the Comite D'Honnem of the Congress. His contribution on " The Agricultural Basis of Religion in India " was received at the Congress with great appreciation. After a successful continental tour, he returned to India in August 1934. Soon after his return to India, he was deputed by the Chief Commissioner of Coorg to undertake the ethnographic survey of that interesting State, and he was supported in his labours by Dr. Cipriani. He had completed his work before February 1936 and the monographs are now ready for publication.

In recognition of his great achievements, the President of France honoured him by electing him to the distinction of the Officer D'Academie. The Government of India conferred on him the title of Dewan Bahadur. The University of Breslau awarded him the Honorary Doctorate of Medicine and Surgery (the Faculty of Medicine controlling the Department of Anthropology and Ethnology in the continental Universities).

It is difficult adequately to sum up within the limits of this brief memoir Dewan Bahadur Dr. Ananthakrishna Iyer's work on Indian Ethnology. The influence of his writings on European Anthropologists seems to have been considerable. The Director of the Anthropological Institute and Ethnographical Museum of Breslau, Baron von Eickstedt, in a communication dated September 23rd, 1930, expressed the general opinion of the European Anthropologists on Dr. Iyer's works in these terms :—

" All are unanimous in that India possesses in you one of the most careful, active, and assiduous scholars of which Ethnology can boast of in any culture or country. Accordingly one finds your name mentioned with appreciation in English, German, Austrian and Italian works. The great text-book of Father W. Schmidt—who first started the connections between the Mundari and Monkhmer peoples—is full of the results of your research work and full of your instructive pictures from Southern India. This is the

recognition of the fact that since long your works are considered to be a real storehouse of cultural and historical knowledge and are highly appreciated. We, therefore, not only honour you, but venerate you as the father of Indian Ethnology....Should there be any scholar really interested in civilisation, history and cultural future of his country, who should not know and respect your name, the name of the father of Indian Ethnology ? Your admirable assiduity, your useful activity, your sound judgment and extensive knowledge are rendering you an ornament to every university, which may be so lucky as to have you in the ranks of her Professors."

The vast output of his scientific investigations and reports was all during the last thirty-six years of his life, and a large part of it done after his retirement from the full term of official service in the Cochin State, a year or two before he attained his sixtieth year. So great a capacity and zeal for a type of work which necessitated frequent travel mostly in out-of-the-way places and a dislocation of his ordinary habits of life is not easily matched.

Dr. Ananthakrishna Iyer's efforts to popularise the subject of Anthropology in the Indian Universities is an important contribution to the progress of scientific education in this country.

When the Indian Academy of Sciences and the National Institute of Sciences of India were founded, Dr. Ananthakrishna Iyer was elected a Fellow of both these Institutions and he acted as President of the Anthropological Section of the Indian Science Congress on five distinct occasions. Dr. Ananthakrishna Iyer will always be remembered by his numerous friends for his great geniality, stern rectitude of character, simple habits of life, unostentatious and unassuming manners. His scholarship was as wide as his powers of exposition were remarkable. He was happy in his domestic life, and his sons have already earnt a great reputation in the respective fields of their activity. Dr. Ananthakrishna Iyer had the most lovable gift of making friends and retaining them. He never offended anyone nor made a single enemy in his long and honourable life.

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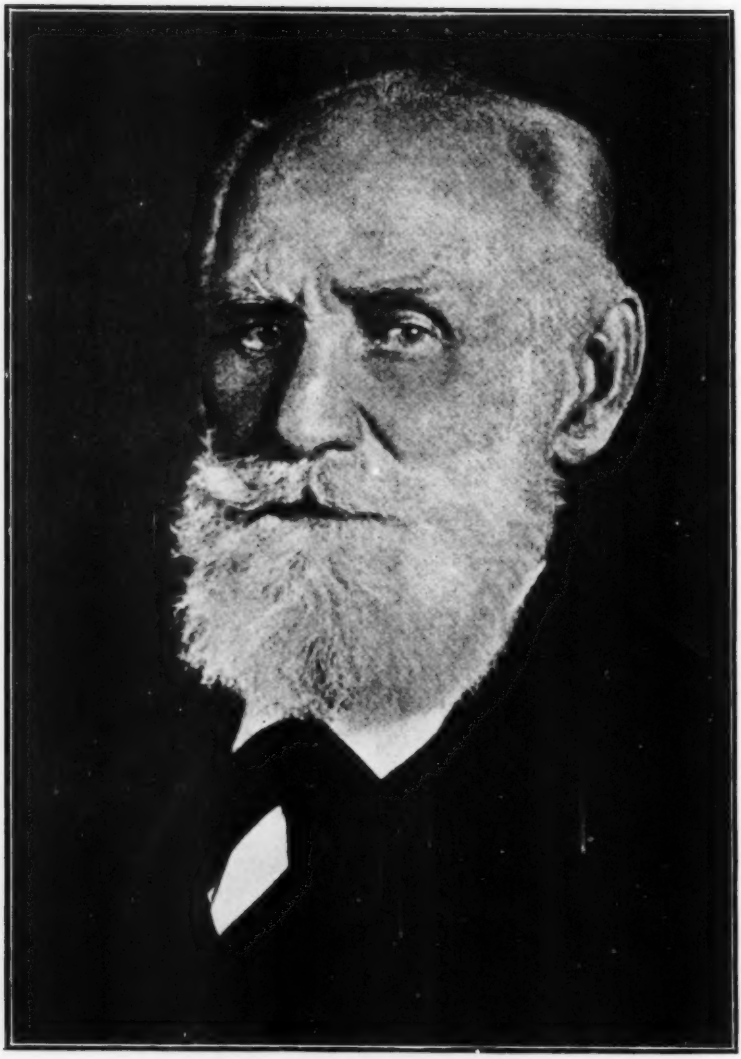
C. R. N.



PROFESSOR IVAN PETROVICH PAVLOV

OBITUARY NOTICE.

Professor I. P. Pavlov.
(1849–1936.)

THE Indian Academy of Sciences has suffered a grievous loss in the death of Professor Pavlov, who was elected one of its Honorary Fellows in 1934. His election to any organisation would confer prestige and the Indian Academy of Sciences was the first Institution in India to confer upon this distinguished scientist the Honorary Fellowship and had the satisfaction of its invitation being gratefully accepted. It is hardly necessary to say that the passing away of this great man at the age of eighty-six has left a void in the circle of scientists, which it will take a long time to fill.

Professor Pavlov was a Nobel Prizeman, having been elected to that distinction in 1904, in recognition of his researches on the functions of the digestive glands. Professor Pavlov was the recipient of numerous honours —he was Foreign Member of the Royal Society (1907), elected Member of the Russian Academy of Sciences (1907), and Honorary Fellow of the Royal College of Physicians (1928). He was awarded the Copely Medal in 1918. As Director of Physiological Laboratories in the Russian Academy of Medicine and the Institute of Experimental Medicine, he attained world-wide fame and carried out extensive investigations on the problems of digestion, of cerebral activity and the theory of reflexes.

786-37—Printed at The Bangalore Press, Mysore Road, Bangalore City, by G. Srinivasa Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore.

Erratum.

Vol. V, No. 5, May 1937.

Page 175, line 33—*for* "non-fragile" *read* "fragile".

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